The Effect of Diverse Forage Species and Irrigation Management on Plant Nitrogen Uptake and Nitrate Leaching Losses

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Anna Jane Carlton

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Reducing the loss of nitrogen (N) through nitrate (NO_3^-) leaching from cow deposited urine patches is one of the greatest challenges facing the New Zealand dairy industry. The objective of this PhD research programme was therefore to determine the effect of diverse forage species and irrigation management on plant N uptake and NO_3^- leaching from the urine patch.

The first experiment was conducted using lysimeters collected from a free-draining Paparua fine sandy loam soil located on the Lincoln University Research Dairy Farm (LURDF), Canterbury, New Zealand. The lysimeters were collected from two pre-existing grazed forages: (i) a 'standard' forage containing perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.); and (ii) a 'diverse forage' containing perennial ryegrass, white clover, red clover (*Trifolium pratense* L.), plantain (*Plantago lanceolata* L.), chicory (*Cichorium intybus* L.) and prairie grass (*Bromus willdenowii* L.). Dairy cow urine was applied to both sets of lysimeters in late spring at a loading rate of either 500 or 700 kg N ha^{-1}. Following the urine application, irrigation was applied at a rate of 18 mm every three days ('optimum') or 9 mm every three days ('deficit') from November to April. The herbage N uptake and N leaching in drainage were measured thereafter using standard methods. Compared with deficit irrigation, NO_3^- leaching losses were 88–97% lower under optimum irrigation at a urine application rate of 700 kg N ha^{-1}. Leaching losses from the 500 kg N ha^{-1} urine treatment were below 4 kg N ha^{-1} for both forage types and there was no significant difference between the irrigation treatments. The differences in NO_3^- leaching losses were attributed to greater herbage growth and N uptake during the summer period by forages that were supplied with sufficient water under optimum irrigation. Forage type had no effect on herbage N uptake or NO_3^- leaching losses when applied at 700 kg N ha^{-1}.

The second and third experiments were conducted using lysimeters and companion soil blocks which were also collected from a free-draining Paparua fine sandy loam soil located on the LURDF. The lysimeters and soil blocks were collected from two pre-existing irrigated plots: (i) a standard forage containing perennial ryegrass and white clover; and (ii) a diverse forage containing perennial ryegrass, white clover and plantain. Dairy cow urine, at a N loading rate of 700 kg N ha^{-1}, was applied to one set of 30 lysimeters in December (early summer). A second set of 30 lysimeters and 24 soil blocks received a dairy cow urine application in February (late summer). Following the urine application, irrigation was applied to all lysimeters from December to April using one of the following systems: (i) pivot irrigation at a rate of 15 mm every three days; (ii) rotorainer irrigation at a rate of 45 mm every nine days; and (iii) flood irrigation at a rate of 90 mm every 18 days. The herbage N uptake and N leaching in drainage from the lysimeters were measured thereafter using standard methods. Soil cores were taken from the soil blocks and were used to measure ammonia oxidizing bacteria (AOB) and archaea (AOA) abundance and nitrification rates under these cow urine patches. Compared with the standard forage, NO_3^- leaching losses were 82% lower under the diverse forage containing plantain when urine was applied in December and 74% lower when urine was applied in February. The companion soil blocks showed that compared with the soil under the standard forage, AOB abundance was lower under the diverse forage containing plantain. Consequently, soil NH_4^+–N concentrations remained higher under the diverse forage while the NO_3^-–N concentrations were lower. The differences in NO_3^- leaching losses were attributed to a combination of nitrification inhibition (likely due to a biological nitrification inhibitor released from the plantain) and reduced drainage losses under the diverse forage. Irrigation type had no effect on herbage N uptake or NO_3^- leaching losses.

In conclusion, the strategic use of diverse forages containing plantain, is a viable mitigation option to reduce NO_3^- leaching losses from urine patch areas. Furthermore, results have demonstrated that diverse forages can perform well under a range irrigation types in New Zealand when irrigation is applied using best management practices.

Keywords: Nitrate leaching, irrigation, diverse forage, perennial ryegrass, plantain, nitrification inhibition, ammonia oxidising bacteria, urine patches, nitrogen uptake, root architecture.

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Chapter 1

Introduction

In New Zealand, dairy farming has intensified and expanded dramatically in the past two decades. Dairy farming is economically important for New Zealand where dairy products contributed to approximately 15% of New Zealand's export revenue in 2016 (Statistics New Zealand, 2016). In parts of New Zealand, the expansion of the dairy sector has occurred due to a rapid expansion of irrigated land. The significant production and economic benefits associated with irrigation, have led to the extensive conversion of dryland areas into dairy and cropping systems, particularly in the Canterbury and North Otago regions (Doak, 2004; McDowell *et al.*, 2011). However, this rapid expansion and intensification of the dairy sector has raised concerns about the impact of nitrate (NO_3^-) leaching on water quality and human health. When the limitation of soil moisture is removed by irrigation, higher nitrogen (N) inputs can be used to obtain maximum herbage production. The additional herbage grown is then often matched by an increase in the number of cows grazed on the farm (De Klein *et al.*, 2010). This becomes an environmental concern because a major source of N lost from grazed systems is N excreted in cow urine (Selbie *et al.*, 2015). Because urine is excreted in patches, rather than evenly distributed across the paddock (Lantinga *et al.*, 1987), the amount of N in the urine patch can exceed plant nutritional requirements. Surplus N (in the form of NO_3^-) is susceptible to leaching when drainage occurs (Cameron *et al.*, 2013). High concentrations of NO_3^- in drinking water are considered a risk to human health where NO_3^-–N concentrations exceed the 11.3 mg L^{-1} limit set by The World Health Organisation (World Health Organisation, 2011). In addition, elevated N concentrations in surface water can lead to eutrophication, thus having a detrimental effect on ecosystem health and recreational activities (Smith & Schindler, 2009).

In 2011, the National Policy Statement for Freshwater Management (NPFWM) came into effect in New Zealand (Ministry for the Environment, 2014). Under the NPFWM, regional councils must set and manage land uses within the water quality limits. In future, this may require substantial changes in typical farm practices to achieve NO_3^- losses below the current levels, and a resource consent may be required to continue an existing land use (Chapman *et al.*, 2014; Ministry for the Environment, 2014). It is therefore critical to develop mitigation strategies which reduce the environmental footprint of agriculture without limiting farm production. Mitigation strategies to reduce NO_3^- leaching losses from grazed systems have been reviewed by several authors e.g. Di and Cameron (2002a), Stark and Richards (2008). More recently, the integration of diverse forages i.e. forages containing grasses, legumes and herbs, has been promoted as an option to reduce NO_3^- leaching losses (Pembleton *et al.*, 2015; Vibart *et al.*, 2016), however the mechanisms behind N loss reductions are poorly understood or quantified. Furthermore, as the area of land under irrigation expands in New Zealand, there is a need to better understand the effect of irrigation management practices on NO_3^- leaching losses and how diverse forages perform under irrigated and water limited grazed systems.

Plants species including the herbs: plantain (*Plantago lanceolata* L.) and chicory (*Cichorium intybus* L.), legumes: lucerne (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.), and grasses: prairie grass (*Bromus willdenowii* L.) and Italian ryegrass (*Lolium multiflorum* Lam.) are among the key species being considered in New Zealand diverse forage mixtures (Pembleton *et al.*, 2015). The inclusion of diverse forages, particularly those containing plantain, in the diet of cows has been shown to reduce the concentration of N in cow urine compared with standard perennial ryegrass (*Lolium perenne* L.) and white clover forages (*Trifolium repens* L.) (Woodward *et al.*, 2012; Totty *et al.*, 2013; Edwards *et al.*, 2015; Box *et al.*, 2016). This is of interest because a reduced N loading rate in the urine patch could have the potential to reduce NO_3^- leaching losses from these N 'hotspots' (Selbie *et al.*, 2015). It is also thought that forage species such as plantain, may release biological nitrification inhibiting compounds into the soil (Dietz *et al.*, 2013; Massaccesi *et al.*, 2015), which could reduce the risk NO_3^- leaching. However, there are no reports of plantain effects on nitrification and subsequent NO_3^- leaching losses from cow urine patches and this represents a gap in the knowledge and an opportunity for mitigation of NO_3^- leaching.

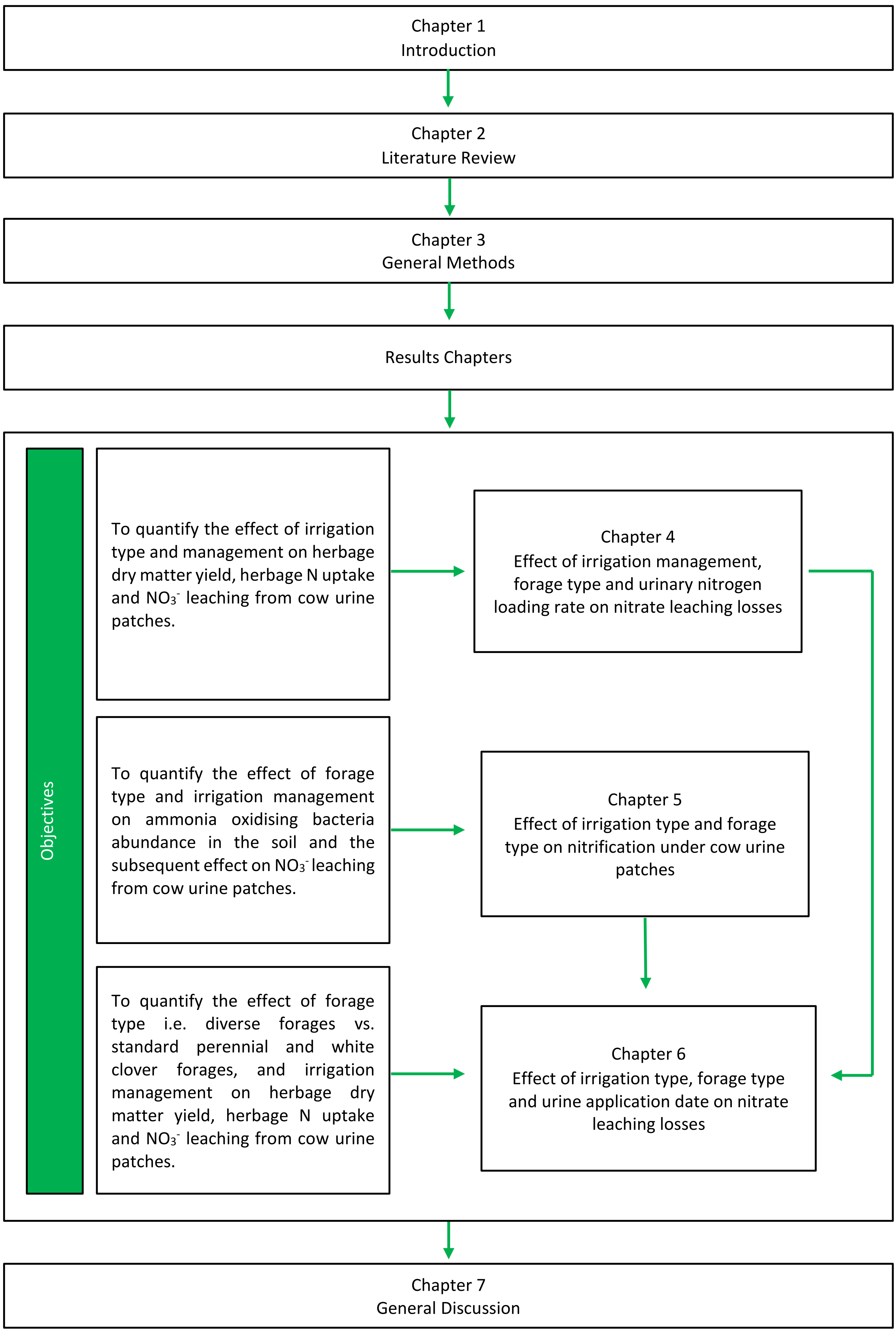
Three main types of irrigation system are used in New Zealand grazed systems: (i) pivot/spray; (ii) rotorainer irrigation; and to a lesser extent (iii) flood irrigation (Irrigation New Zealand, 2017). Previous studies have shown than high irrigation volumes typically correspond with a greater leaching depth and increased NO_3^- leaching losses due to excess water moving through the soil (Moore, 2002; Daudén *et al.*, 2004). Once leached below the root zone, the nutrients can no longer be taken up and there is the potential to contaminate ground and/or surface water. Field and glasshouse studies have indicated that certain forage species might have an effect on N interception and/or NO_3^- leaching due to differences in root architecture and niche separation (Crush *et al.*, 2005; Skinner & Comas, 2010; Moir *et al.*, 2013; Malcolm *et al.*, 2014). This may also give diverse forages an advantage in both irrigated and water limited systems (Neal *et al.*, 2009; Nobilly *et al.*, 2013). Of key interest is how diverse forages respond to irrigation and whether they can be used to increase N capture and subsequently reduce NO_3^- leaching losses.

Therefore, the overall objectives of this PhD research project were:

- To quantify the effect of irrigation type and management on herbage dry matter yield, herbage N uptake and NO_3^- leaching from cow urine patches.

- To quantify the effect of forage type i.e. diverse forages vs. standard perennial and white clover forages, and irrigation management on herbage dry matter yield, herbage N uptake and NO_3^- leaching from cow urine patches.

- To quantify the effect of forage type and irrigation management on ammonia oxidising bacteria abundance in the soil and the subsequent effect on NO_3^- leaching from cow urine patches.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Literature review

2.1 The New Zealand dairy industry

Dairy farming in New Zealand has intensified and expanded substantially in the past two decades, with dairy product exports contributing to 15% of New Zealand's export revenue in 2016 (Statistics New Zealand, 2016). Dairy production therefore brings large benefit to the national economy. An abundance of suitable agricultural land, favourable climatic conditions and unique farming practices have facilitated the rapid increase in dairy production. At the end of the 2015/16 season, there were 1.75 million effective hectares being used for dairy production (New Zealand Dairy Statistics, 2016). In drier areas, such as Canterbury and North Otago, the significant production and economic benefits associated with irrigation have led to the extensive conversion of dryland areas into dairy and dairy support systems (Doak, 2004; McDowell *et al.*, 2011). This has led to cow numbers surpassing five million in 2015, a 44% increase from the early 2000's (New Zealand Dairy Statistics, 2016).

In New Zealand, grazed systems are typically based on temperate forage species. The most common forage used in New Zealand is a mixture containing perennial ryegrass (*Lolium perenne* L.) and white cover (*Trifolium repens* L.) (Vibart *et al.*, 2016). Dairy cows are typically grazed on a perennial ryegrass and white clover forage for the majority of the year, with fodder crops (e.g. turnips [*Brassica rapa*] and kale [*Brassica oleracea*]) and supplementary feeds (e.g. baleage) utilised over the winter and summer periods to fill feed deficits (Bryant *et al.*, 2010). The widespread use of perennial ryegrass and white clover forages has been associated with the high nutritious value, quick establishment, high productivity and well understood management requirements of both forage species (Fulkerson & Donaghy, 2001). However, the high protein content of perennial ryegrass and white clover forages can result in greater nitrogen (N) in the feed than is required for milk production. As a result, excess N can be excreted in cow urine patches (Pacheco *et al.*, 2010). This is of concern as N loss (in the form of nitrate [NO_3^-]) from cow urine patches has been linked to water quality issues in New Zealand (Di & Cameron, 2002a).

2.2 The urine patch in grazed systems

Nitrogen excreted from grazing dairy cattle plays a key role in N cycling through pastoral systems (Haynes & Williams, 1993). Urine deposition by grazing animals results in 'hot spots' of high N loading compared to the surrounding pasture and soil. These appear in the paddock as visible patches of denser, darker green pasture compared with the surrounding pasture (Plate 2.1) (Moir *et al.*, 2011).

Plate 2.1. Urine patches deposited by grazing dairy cows (highlighted in red).

The urine patch can be defined as the wetted area (the 'wet' area directly where urine was deposited) or as the effective area. The effective area encompasses both the wetted area and surrounding area affected by urinary–N via urine diffusion, NH_3 deposition and plant root extension (Lantinga *et al.*, 1987; Buckthought *et al.*, 2016). A *meta*-analysis of published studies by Selbie *et al.* (2015) found the wetted area of a single urination event was on average 0.24 m^2. However, the effective area has been reported to range from 0.03 to 1.1 m^2 (Lantinga *et al.*, 1987; Moir *et al.*, 2011; Dennis *et al.*, 2013; Buckthought *et al.*, 2016).

It is estimated that cow urine patches can cover 10–30% of a grazed paddock area (4 year trial period) (Moir *et al.*, 2011). However, this can vary with farm stocking rates and the frequency of urination events (Haynes & Williams, 1993; Silva *et al.*, 1999; Franzluebbers *et al.*, 2000; White *et al.*, 2001; Dennis *et al.*, 2011; Moir *et al.*, 2011; Dennis *et al.*, 2013). In a urination behaviour study of dairy cows Draganova *et al.* (2010) found 85% of the total urinations occurred on pasture. The remaining 15% were deposited along the races, in the holding yards or in the milking shed.

The volume of urine produced in single urination event is also highly variable and reported to range from 1.6–2.4 L per urination event (Doak, 1952; Haynes & Williams, 1993; Betteridge *et al.*, 2013; Beukes *et al.*, 2014; Edwards *et al.*, 2015; Ravera *et al.*, 2015). Betteridge *et al.* (2013) reported large variability between individual animals with reported volumes ranging from 0.30 L to 7.83 L per urination event over an 11–day trial in August 2012.

The urinary–N concentration is reported to range from 2.0–20.0 g N L^{-1}. Assuming a 2 L urination volume and a surface area of 0.2 m^2, this is equivalent to N loading rates in the urine patch of 200–2000 kg N ha^{-1} (Selbie *et al.*, 2015). The primary factor affecting the amount of N excreted in cow urine is the N content of the feed (Haynes & Williams, 1993). Typically, as the N content increases, the amount of N excreted in the urine increases (Castillo *et al.*, 2000; Yan *et al.*, 2007). However, water intake has also been shown to reduce urinary–N concentrations through dilution effects (Cheng *et al.*, 2017), thus a high N diet may not always correspond with a high urinary–N concentration. The effect of forage type on cow urinary–N excretion and subsequent N loading rates is discussed further in Section 2.5.1.

2.3 Nitrate leaching from the urine patch

The term 'leaching' refers to the movement of N down the soil profile via drainage water. The amount of N deposited into the soil from a urine patch, soil N transformations, plant N uptake, and the amount of drainage that occurs through the soil are the key processes which govern the amount of NO_3^- leached from the soil (Cameron *et al.*, 2013). Climate, soil properties and farm management practices can also affect NO_3^- leaching, and are discussed in greater detail by Di and Cameron (2002a) and Cameron *et al.* (2013). A *meta*-analysis by Selbie *et al.* (2015), reported that the amount of NO_3^-–N leached from cow urine patches ranged from 40 to 629 kg N ha^{-1} from cows grazing pasture.

2.3.1 Nitrogen loading rate

The N loading rate of cow urine patches has been reported to range from 200–2000 kg N ha^{-1} (Selbie *et al.*, 2015). A mismatch between the N content of feed and cow dietary requirements has led to a surplus of N to be excreted in the urine. The N content of forages consumed on a typical New Zealand farm have been reported to range from 2.8–4.5% (Ledgard *et al.*, 2007). It has been suggested that when the N content of feed exceeds 3.2% (20% crude protein), N can become surplus to requirements (Pacheco *et al.*, 2010). Depending on the feed source, this can result in 60–90% of the N consumed by the cow being returned to the soil. Approximately, 70% of this is deposited as urine (Haynes & Williams, 1993). Because urine is excreted in patches, rather than evenly distributed across the paddock, the amount of N in the urine patch can exceed plant nutritional requirements (Lantinga *et al.*, 1987). Surplus N (in the form of NO_3^-) is therefore susceptible to leaching when drainage occurs. Di and

Cameron (2007) reported greater NO_3^- leaching losses with increasing N loading rates. For example, the total amount of NO_3^- leached was measured to be 4.3 times greater at a loading rate of 1000 kg N ha^{-1} compared to a loading rate of 300 kg N ha^{-1} (Figure 2.1).

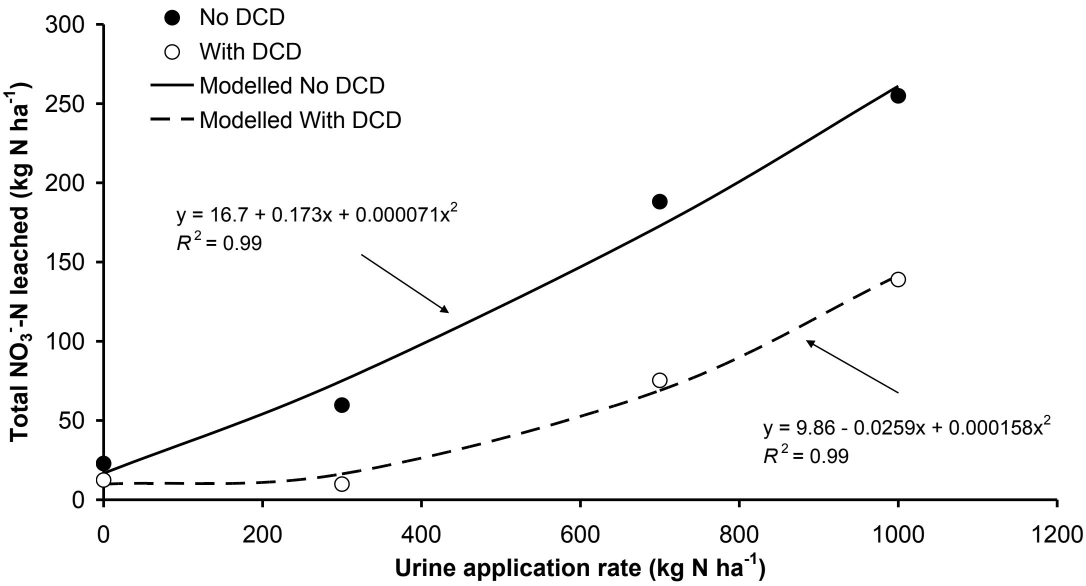


Figure 2.1. Relationships between urine application rate and total NO_3^--N leaching loss with and without DCD applied (Di & Cameron, 2007).

2.3.2 Nitrification

Nitrification, a microbial process mediated by ammonia oxidising bacteria (AOB) and archaea (AOA), determines the amount of NO_3^- present in the soil, and therefore how N is utilised or dispersed into the environment (Cameron *et al.*, 2013) (Figure 2.2). A more detailed description of the factors affecting soil nitrification rates can be found in Sahrawat (2008).

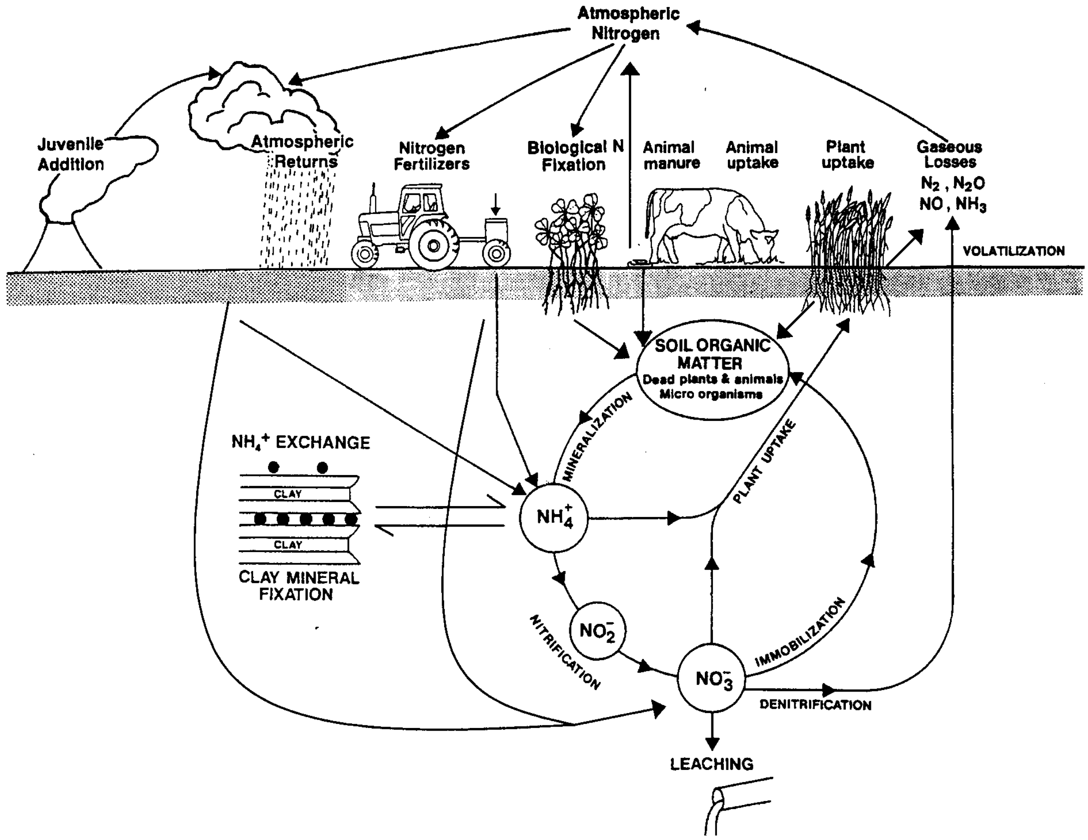


Figure 2.2. The soil/plant nitrogen cycle (Cameron *et al.*, 2013).

Nitrification involves the oxidation of ammonium (NH_4^+) firstly to nitrite (NO_2^-) and then to NO_3^- as described by Equations 1 and 2. The second equation typically occurs rapidly resulting in little accumulation of NO_2^- in the soil.

$$2NH_4^+ \ + \ 3O^2 \ \rightarrow 2NO_2^- \ + \ 2H_2O + \ 4H^+ \ + energy \tag{1}$$

$$2NO_2^- + \ O_2 \ \rightarrow \ 3NO_3^- + energy \tag{2}$$

In N-rich environments, such as grazed forage systems, nitrification is mainly conducted by AOB including *Nitrosomonas, Nitrosospira* and *Nitrobactor.* The first reaction is controlled by the ammonia monooygenase enzyme associated with *Nitrosomonas* and *Nitrosospira* bacteria. The second reaction is primarily controlled by *Nitrobactor* bacteria (Di *et al.*, 2009; Cameron *et al.*, 2013). Studies have shown that increases in AOB abundance are linked to nitrification activity following amendment with high levels of NH_4^+ (Di *et al.*, 2009; Jia & Conrad, 2009; Di *et al.*, 2010; Verhamme *et al.*, 2011; Parfitt *et al.*, 2012). For example, the number of AOB present in the soil has been shown to increase 3.2 to 10.4 fold in response to a urinary–N application (Di *et al.*, 2009).

Large numbers of AOA are also present in the soil (Leininger *et al.*, 2006). However, AOA abundance has not been shown to increase in response to high levels of soil NH_4^+, suggesting that in grazed systems, AOA are not the key drivers of nitrification (Di *et al.*, 2009). In contrast, an increase in AOA abundance has been associated with nitrification in soils with a continual supply of NH_4^+ at low concentrations (Offre *et al.*, 2009). This is supported by the findings of Di *et al.* (2010), who observed higher AOB abundance in a N rich topsoil, whereas AOA were more abundant in the subsoil. Studies have also shown AOA to contribute to nitrification in marine environments, low N grassland systems (Leininger *et al.*, 2006) and acidic soils (Yao *et al.*, 2011; Zhang *et al.*, 2012).

2.3.3 Solute movement and drainage

Drainage occurs when soils approach or exceed field capacity, i.e. when there is excess rainfall over evapotranspiration. Because NO_3^- is an anion, it is repelled by cation exchange sites in the soil, thus making it mobile in the soil and readily leached (McLaren & Cameron, 1996). In New Zealand, this typically occurs during the late autumn and winter months when plant growth is low. Alternatively, the preferential flow of water through soil macropore networks can also facilitate rapid drainage and the movement of NO_3^- when large volumes of water are applied to the soil surface at a rate which exceeds soil matrix infiltration. Leaching is generally minimal in summer; however, it may occur via macropore flow under heavy rainfall events or inefficient irrigation systems (Cameron *et al.*, 2013).

Nitrate in drainage water can be transported through the soil via a combination of three primary mechanisms: (i) convection; (ii) diffusion; and (iii) hydrodynamic dispersion (Cameron *et al.*, 2013). *Convection* occurs when dissolved NO_3^- moves with the mass flow of water in the soil during drainage events as described by Equation 3.

$$J_c = qc = -c\left(K\frac{dH}{dH}\right) \tag{3}$$

Where J_c is the convective NO_3^- flux, c is the concentration, q is the water flux, K is the hydraulic conductivity and dH/dx is the hydraulic gradient. However, the water and NO_3^- do not travel uniformly through the soil, but tend to spread throughout the soil via the processes of diffusion and hydrodynamic dispersion.

Diffusion occurs where the uneven distribution of NO_3^- in solution results in a concentration gradient, and consequently the movement of NO_3^- from highly concentrated areas to areas of lower concentration as describe by Equation 4.

$$J_d = -\left(D_s \frac{dc}{dx}\right) \quad (4)$$

Where J_d is the rate of diffusion, D_s is the diffusion coefficient of nitrate in the soil and depends on the soil moisture content and *dc/dx* is the nitrate concentration gradient.

Hydrodynamic dispersion occurs due to the mixing of the solute by the mechanical action of water flow through the soil. This is due to the heterogeneous nature of the soil, namely the large variation in pore size and thereby pore water velocities, and by the tortuosity of soil pores generating an array of flow paths. Collectively, these NO_3^- transport mechanisms are termed as *'combined convective-diffusive-dispersive transport'* as described in Equation 5.

$$\frac{\partial c}{\partial t} = Da\frac{\partial^2 c}{\partial x^2} - U\,\frac{\partial c}{\partial x} \quad (5)$$

Where D_a is the apparent diffusion coefficient and represents the sum of molecular diffusion plus hydrodynamic dispersion.

2.4 Environmental impact of nitrate leaching

Water quality is now considered one of the important environmental issues facing New Zealand. There is increasing concern surrounding the impact of low water quality on a range of economic sectors including recreational use, drinking water, energy security, and irrigation requirements (Marsh, 2012; Duncan, 2014). Approximately 40% of New Zealand's population relies on groundwater for drinking, thus elevated NO_3^-–N in groundwater is a potential threat to human health (Rajanayaka *et al.*, 2010). High concentrations of NO_3^- in drinking water are considered a risk to human health where NO_3^-–N concentrations exceed the 11.3 mg L^{-1} limit set by The World Health Organisation (World Health Organisation, 2011). The health risks associated with groundwater levels of NO_3^-–N that exceed the maximum acceptable level for drinking water include methaemoglobinaemia ("blue baby syndrome") in young infants and increased risk of stomach cancer and heart disease in adults (World Health Organisation, 2011). Water high in NO_3^-–N can also be toxic to livestock at concentrations ranging from 40–100 mg NO_3^-–N L^{-1} where it can result in livestock methaemoglobinaemia, fatality and abortions (Di & Cameron, 2002a). Furthermore, many New Zealand surface waters are N and/or P limited, thus the introduction of excess N can lead to an increase in ecosystem productivity resulting in algal blooms or accelerated eutrophication. When these aquatic plants and/or algae die, their decomposition depletes the water of dissolved oxygen, consequently changing the ecosystem dynamics (McLaren & Cameron, 1996; Smith & Schindler, 2009). Recent reviews by Davies-Colley (2013), Parkyn and Wilcock (2004) and Quinn *et al.* (2009) have consistently reported that intensification of agriculture is associated with increasing nutrient levels in streams on a national scale.

It is now widely accepted that agriculture contributes to declining water quality in New Zealand (Monaghan *et al.*, 2007). Due to the importance of freshwater quality in New Zealand, the National Policy Statement for Freshwater Management (NPSFM) came into effect in New Zealand in 2011. The aim of the NPSFM was to set objectives and policies for regional councils to manage water in an integrated and sustainable way, while providing for economic growth within set water quantity limits (Ministry for the Environment, 2014). In future, this may require substantial changes in typical farm practices to achieve NO_3^- losses below the current levels, and a resource consent may be required to continue an existing land use (Chapman *et al.*, 2014).

It is therefore critical that mitigation strategies are developed to reduce the environmental footprint of agriculture without limiting farm production. Mitigation strategies to reduce NO_3^- leaching losses from grazed systems have been reviewed by several authors e.g. Di and Cameron (2002a), Stark and Richards (2008). Recently, there has been increased interest in the use of diverse forages and irrigation management tools to reduce NO_3^- leaching losses. These are discussed in greater detail below.

2.5 Diverse forages to mitigate nitrate leaching

Diverse forages (containing three or more plant species), potentially offer a feed based strategy to reduce NO_3^- leaching from grazed forages. Plants species including the herbs; plantain (*Plantago lanceolata* L.) and chicory (*Cichorium intybus* L.), legumes; lucerne (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.), and grasses; prairie grass (*Bromus willdenowii* L.) and Italian ryegrass (*Lolium multiflorum* Lam.) are among the key species being considered in New Zealand. Diverse forages could potentially reduce NO_3^- leaching losses from the urine patch in a number of ways. These may include reducing the concentration of N excreted in the urine, increasing the ability of plants to capture N in the soil profile, or by inhibiting nitrification through the release of secondary plant compounds (Pembleton *et al.*, 2015; Vibart *et al.*, 2016).

2.5.1 Urinary–N excretion

The concentration of N in urine is an important component of N loading rates and the resulting risk of NO_3^- leaching losses from urine patches (Li *et al.*, 2012). Recently, increased interest has emerged around the role of diverse forages in reducing the concentration of N excreted in cow urine Edwards *et al.* (2015). Studies have shown that cows fed a diet containing grasses, legumes and herbs can reduce the urinary–N excretion in dairy cows without comprising milk production (Woodward *et al.*, 2012; Totty *et al.*, 2013; Edwards *et al.*, 2015; Box *et al.*, 2016; Bryant *et al.*, 2017; Cheng *et al.*, 2017).

Results from Totty *et al.* (2013) showed that when cows were grazing a diverse forage (perennial ryegrass, white clover, chicory, plantain, red clover, lotus [*Lotus pedunculatus*] and prairie grass), urinary–N excretion was lower compared with cows grazing a standard perennial ryegrass and white clover forage (353.8 vs. 438.3 g N cow day^{-1}). While the diverse forage provided a lower N diet, the differences in N intake did not fully explain the reduction in urinary–N that was observed. It was thought that the presence of secondary plant metabolites such as tannins altered the partitioning of N between the urine and faeces. An indoor trial by Woodward *et al.* (2012) also reported that lactating dairy cows fed a diverse forage partitioned more N into milk than cows fed on a standard perennial ryegrass and white clover forage (23% vs. 15%). In the same trial, urinary–N excretion was halved in the cows fed the diverse forage (2.6 vs. 6.2 g N L^{-1}).

There is increasing evidence which suggests that the incorporation of plantain into the diet has a key role in reducing urinary–N excretion (Edwards *et al.*, 2015; Box *et al.*, 2016; Cheng *et al.*, 2017). This is thought to be through a combination of factors including; the lower water soluble carbohydrate concentration of plantain (Edwards *et al.*, 2007); diuretic effects; secondary plant compounds; or increased water intake due to the lower dry matter (DM) content of plantain. For example, Cheng *et al.* (2017) found that cows consuming plantain had a lower urinary–N excretion than those grazing perennial ryegrass and white clover forage (47 vs. 70 g N day^{-1}). This was attributed to the greater total water intake by cows fed plantain (1.3 times higher) and improved N use efficiency. Similarly, Box *et al.* (2016) measured a 50% and 33% reduction in the urinary–N concentration of cows fed 100% plantain or a 50-50 pasture-plantain forage when compared with perennial ryegrass and white clover alone. Furthermore, when modelling the potential for diverse forages to reduce NO_3^- leaching losses on a farm scale, Beukes *et al.* (2014) reported that when 50% of the farm was sown in a diverse forage, a reduction in urinary–N concentration and a dilution effect from large volumes of urine could potentially reduce NO_3^- leaching by 19%. These studies suggest that diverse forages containing plantain could be a viable option to reduce the environmental impact of dairy farming. However, field experiments are now needed to directly quantify the effect that urine deposited from cows grazing diverse forages has on NO_3^- leaching losses.

2.5.2 Nitrogen capture and DM production

Field and glasshouse studies have indicated that certain forage species can have an effect on N interception and/or NO_3^- leaching losses due to differences in root architecture and niche separation (Crush *et al.*, 2005; Skinner & Comas, 2010; Moir *et al.*, 2013; Malcolm *et al.*, 2014). This may give diverse forages an advantage in both irrigated and water limited systems (Neal *et al.*, 2009; Nobilly *et al.*, 2013). Perennial ryegrass and white clover forages are characterised by a shallow rooting system, with most roots occurring in the top 15 cm of soil (Haynes & Williams, 1993). However, depending on the plant species used, diverse forages based on perennial species tend to have a greater rooting depth than standard forages (Skinner *et al.*, 2004; Skinner *et al.*, 2006). Because ryegrass rooting depths are typically low in the subsoil, increasing root growth in this region may allow herb species to exploit different niches for water and N. This in turn could increase both water and N uptake thereby reducing the risk of NO_3^- leaching from the soil (Wiesler & Horst, 1993; Wiesler & Horst, 1994; Sanderson *et al.*, 2004). For example, Wiesler and Horst (1994) found that the subsoil root length of field grown maize had a positive correlation with N uptake and a negative correlation with NO_3^- leaching. Modelling work by Snow and White (2013) has also shown that increasing root depth has the potential to increase N capture from cow urine patches. While there was initially no difference in N uptake, after 180 days the results indicated that forages with a deeper root system had greater growth rates, resulting in a decrease in soil mineral N. However, in contrast to these results, other studies have suggested soil N interception was greater in plant species with finely divided root structures and a larger surface area (Habib & La Folie, 1991; Dunbabin *et al.*, 2003; Crush *et al.*, 2005). For example, modelling work by Dunbabin *et al.* (2003) found that root density was the key to obtaining lower amounts of NO_3^- leaching (Figure 2.3). This was attributed to the ability of highly branched architectures to quickly capture and deplete the soil of mineral N before large rainfall events occurred. Field experiments that quantify the root architecture of diverse forages containing grasses, herbs and legumes vs. standard perennial ryegrass and clover forages are therefore required to gain a better understanding of the role root architecture could have in the capture of N from lower soil layers and its subsequent effects on NO_3^- leaching losses.

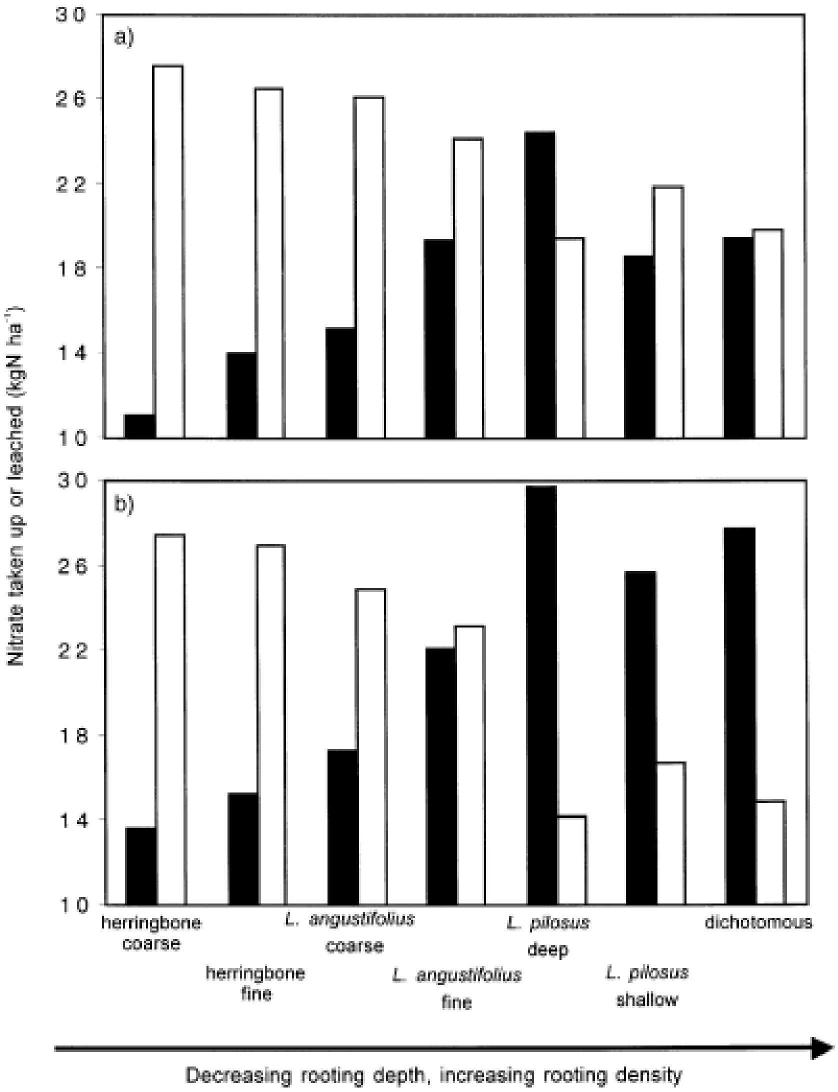


Figure 2.3. Total nitrate taken up (closed bars, kg N ha^{-1}) and total nitrate leached (open bars, kg N ha^{-1}) over the 108 simulation days by seven root architectures. Two rainfall distributions were applied; the 1995 distribution with high rainfall early in the season (a) and the same total rainfall but with the main leaching events delayed until later in the season (b) (Dunbabin et al., 2003).

A study by Skinner *et al.* (2004) measured an 89% increase in dry matter yield under drought conditions when a Kentucky bluegrass (*Poa pratensis*) and white clover forage had three addition species (chicory, perennial ryegrass and orchard grass [*Dactylis glomerata*]) added to the mixture. Greater summer DM production has also been reported by Nobilly *et al.* (2013) when standard perennial ryegrass and white clover forages were sown with additional deep rooting species such as chicory, plantain and lucerne. This was attributed to the adaption of these species to summer water deficits. Studies comparing DM production between two species forages, and those containing greater than two plant species have identified a range of results from no significant increase in DM production to substantial increases. A summary of these studies is presented in Table 2.1, and indicate that there may be an opportunity for alternative forage species to increase N capture during certain times of the year i.e. during summer.

Table 2.1. Effect of standard forages (two species) versus diverse forages (three or more species) on annual herbage DM yield. Adapted from Vibart *et al.* (2016).

Location	Trial duration (year)	Standard forage species	Diverse forage species	Annual herbage mass (t DM ha^{-1})	
				Standard	**Diverse**
Waikato, NZ	4	PR* WC**	WC, tall fescue, chicory, orchardgrass	16.8	20.6
Manawatu, NZ	4	PR, WC	PR, WC, phalaris	16.4	18.2
Pennsylvania, US	2	Orchardgrass, WC	Orchardgrass, WC, chicory, tall fescue, PR, red clover, birdsfoot trefoil, alfalfa, Kentucky bluegrass	7.1	8.8
Terang, AUS	3	PR, WC	WC, tall fescue, orchardgrass, red clover, chicory	9.3	11.7
Naringal, AUS	3	PR, WC	WC, tall fescue, red clover, chicory	10.5	9.8
Waikato, NZ	1	PR, WC, tall fescue	PR, tall fescue, red clover, chicory	13.1	12.4
Waikato, NZ (irrigated)	2	PR, WC, tall fescue	PR, tall fescue, red clover, chicory	17.1	16.4
Canterbury, NZ (irrigated)	2	PR, WC	PR, WC, red clover, prairie grass, chicory, plantain	16.0	16.8
Waikato, NZ	3	PR, WC	PR, WC, prairie grass, chicory, plantain, alfalfa	15.3	14.7

*Perennial ryegrass; **white clover

It is also possible that the characteristics of individual plant species rather than a higher amount of diversity is the critical factor in reducing NO_3^- leaching losses. This appeared to be the case in a study by Malcolm *et al.* (2014) which found that the higher winter activity, and thus daily winter N uptake of Italian ryegrass was more important than root architecture in reducing NO_3^- leaching from autumn deposited urine patches when compared with a standard perennial ryegrass white clover forage. Similarly, Woods *et al.* (2016) found that compared with a perennial ryegrass and white clover forage (205 kg N ha^{-1}), N leaching losses were 35.3% lower from Italian ryegrass (133 kg N ha^{-1}) and 98.5% higher from lucerne (407 kg N ha^{-1}). The lower losses under the Italian ryegrass forage was again attributed to greater cool season activity.

2.5.3 Biological nitrification inhibition

Nitrification inhibition with commercially produced nitrification inhibitors applied to urine treated soil has been well documented (Di & Cameron, 2016). There is now growing evidence which suggests that certain plant species, capable of producing secondary plant compounds, can also suppress nitrifying microbes in the plant root rhizosphere and surrounding soil. The term given to describe this is biological nitrification inhibition (BNI) (Subbarao *et al.*, 2006). The mechanism governing nitrification inhibition is the release of these secondary plant compounds which block the ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO) ammonia oxidizing enzymatic pathways of nitrifying microbes. This results in a delay in the transformation of NH_4^+ to NO_3^- (McCarty, 1999; Zakir *et al.*, 2008; Subbarao *et al.*, 2009). Biological nitrification inhibition has been observed under several plant species including the tropical forage grass *Brachiaria humidicola*, perennial grass *Hyparrhenia diplandra* and *Brassica* species (Lata *et al.*, 2000; Subbarao *et al.*, 2007a; Brown & Morra, 2009; Subbarao *et al.*, 2009). The role of BNI in regulating nitrification in agricultural systems has been reviewed in detail by (Subbarao *et al.*, 2012).

Recent studies have reported the occurrence of nitrification inhibition when the forage species plantain or plantain derived compounds are present (Rauber *et al.*, 2008; Dietz *et al.*, 2013; Massaccesi *et al.*, 2015). For example, the application of aucubin, a secondary plant compound found in plantain, resulted in lower NO_3^- accumulation and higher NH_4^+ accumulation in the soil compared to the controls (Dietz *et al.*, 2013). Similarly, Massaccesi *et al.* (2015) observed lower rates of nitrification from soils under a plantain and grass mixture. The rates of nitrification were observed to increase as the abundance of plantain in the mixtures decreased (Massaccesi *et al.*, 2015). A review by Gardiner *et al.* (2016), also described to the potential for compounds such as aucubin to be excreted in the urine of cows fed a diet containing plantain. It may therefore be possible that compounds excreted in the urine as well as those released into the soil by the plant roots could have a role in nitrification inhibition in diverse forage soils.

To date, most studies investigating BNI activity have been carried out in low N environments. Recent work by Byrnes *et al.* (2017) measured greater nitrification, denitrification and AOA abundance suppression from urine patches under the tropical forage grass *Brachiaria humidicola* cv. Tully (high BNI capacity) when compared with the tropical forage grass *Brachiaria hybrid* cv. Mulato (low BNI capacity). As a result, nitrous oxide emissions were lower from urine patches under the grass *Brachiaria humidicola.* This provides evidence that plant species capable of producing nitrification inhibiting compounds (high BNI capacity) could have a role in reducing N losses from high N environments such the urine patch. However, there is a critical research gap with no direct measurement of the effect of plantain on nitrification rates in high N environments.

2.6 Irrigation to mitigate nitrate leaching

The area of land under irrigation in New Zealand is increasing, particularly as dairy intensification has expanded into drier regions such as Canterbury and Central Otago. As much as 70% of water consented for abstraction in New Zealand is used for irrigation. The current area under irrigation stands at 800,000 ha, with approximately 47% being used for dairy or dairy support (Irrigation New Zealand, 2017). Irrigation in New Zealand is estimated to contribute $2.4 billion to the GDP (farm gate value) (Irrigation New Zealand, 2017). The aim of irrigation is to supply water at times of the year when rainfall is unreliable or inadequate. Irrigation adds value by making up the water deficit over drier periods which enables production to be maximised throughout the season (Irrigation New Zealand, 2017). For example, McBride (1994) measured an increase in herbage yields from 6.7 to 11.9 t DM ha^{-1} when flood irrigation was applied, and Goh and Bruce (2005) found that irrigation doubled the herbage DM yield compared with that of a dryland treatment. Although irrigating forages can be an effective response to drought, applying inadequate water can result in lower forage production (Neal *et al.*, 2009). Moreover, by applying water too quickly, soils may not retain water due to preferential flow down cracks and large pores (Fraser *et al.*, 1994; Cichota *et al.*, 2016), and applying water in excess, can result in nutrients such as N (in the form of NO_3^-) being leached (Gheysari *et al.*, 2009).

Optimum rates of irrigation have been found to increase N uptake by crops and decrease NO_3^- leaching losses (Hahne *et al.*, 1977). However, there is no data reported on the effect of irrigation on N uptake and NO_3^- leaching from diverse forages.

2.6.1 Irrigation types

There are a range of irrigation types used in New Zealand including surface or flood irrigation, travelling irrigators, liner and centre pivots, solid set irrigation, micro-irrigation, and spray-line irrigation (Table 2.2). A detailed summary of the types of irrigation used in New Zealand can be found in (Irrigation New Zealand, 2017).

Table 2.2. Common irrigation systems used in New Zealand.

Type of system	Key characteristics	Level of control	Typical app. depth (mm)	Typical return period	Examples
Surface or flood irrigation	• High application depth and long return periods • Restricted application depth	Low	80-120	2-4 weeks	Border-dyke Wild flooding
Travelling irrigators	• Moderate application depth and return periods • Restricted application depth	Moderate	35-50	9-14 days	Rotary booms Guns K-lines
Fixed point spray irrigators	• Small application depths and short return periods • Variable application depths	High	5-25	1-7 days	Centre pivots, drip irrigation

Flood irrigation typically adds water at levels in excess of plant requirements for a brief period of time. The most commonly used form of flood irrigation in New Zealand is the border-dyke system. The border-dyke irrigation method is characterised by a series of parallel ridges separating long, narrow strips which are flooded when a series of gates in the head-race are opened. Under the border-dyke method, the area closest to the gates receives a greater amount of water to ensure the more distant areas of the paddock get an adequate supply (McIndoe, 2001; Irrigation New Zealand, 2017).

Common *travelling irrigators* include gun, fixed boom or rotating boom irrigators such as the 'rotorainer'. Rotary booms, like the rotorainer, are still commonly used to irrigate grazed forages in New Zealand. Rotating booms are connected to a hydrant and move across a field sequentially, strip by strip using a ratchet system that slowly winches the irrigator in. Although there is some scope for changing application depths by changing travel speeds, they are less flexible than pivot irrigators which allow for greater control over application depth (McIndoe, 2001; Irrigation New Zealand, 2017).

Pivot or spray irrigation is now the most commonly used method of irrigation in New Zealand, particularly in grazed systems. In contrast to flood irrigation, pivot irrigation applies less water at more frequent intervals allowing more time for plant uptake, giving a greater water use efficiency than other irrigation types. Pivots have a main pipeline supported above the field by a series of A-frame towers driven by wheels at the base. Water is discharged under pressure from sprinklers mounted along the pipeline as the pivot circulates around the paddock from a fixed point (Plate 2.2). An increase in the adoption of pivot irrigation has stemmed from low labour requirements and a greater flexibility in water application rates allowing farmers to tailor applications to crop requirements thereby maximising production (McIndoe, 2001; Irrigation New Zealand, 2017).

Plate 2.2. Centre pivot irrigator operating on a New Zealand dairy farm.

2.6.2 Effect of irrigation on nitrate leaching from the urine patch

Historically, most studies measuring NO_3^- leaching losses from the urine patch have focused on urine deposited during the autumn period (Selbie *et al.*, 2015). However, exploratory modelling by Vogeler *et al.* (2010) suggested that under dryland conditions, NO_3^- leaching losses from summer deposited cow urine patches may also be substantial. Summer droughts leading to low plant N uptake, and occasional summer rains leaching NO_3^- below the root zone were considered the key factors contributing to the high NO_3^- leaching losses that were modelled from summer deposited urine patches. Similarly, Scholefield *et al.* (1993) measured twice as much leaching after a hot dry summer than a cool wet one in a seven year lysimeter trial grazed by beef cattle. Increasing plant N uptake from spring and summer deposited cow urine patches through irrigation or improved irrigation scheduling may therefore have the potential to reduce NO_3^- leaching losses from cow urine patches in grazed systems.

The potential for irrigation to reduce NO_3^- leaching losses has been demonstrated by Snow and White (2013) using the process based model APSIM (Agricultural Production Systems Simulator). In this study, cow urine (750 kg N ha^{-1}) was simulated to be deposited in spring onto dryland pasture. The model showed that as water inputs increased, an additional 1.1 t DM ha yr^{-1} was grown, and as a result, soil mineral N was reduced by 118 kg N ha^{-1} six months after urine deposition. It was suggested that by increasing growth rates in summer, potentially leachable mineral N in the soil could be reduced prior to the onset of drainage later in the season. Several cropping studies have also shown that NO_3^- leaching can be reduced under good irrigation management (Hahne *et al.*, 1977; Groves & Bailey, 1997; Paramasivam *et al.*, 2001; Gheysari *et al.*, 2009). For example, Hahne *et al.* (1977) found that the optimum irrigation of a corn crop reduced N leaching losses from 48% to 5% of the N applied. Optimum irrigation has also been shown to reduce residual soil N by 31 kg N ha^{-1} compared with an unirrigated treatment (79 kg N ha^{-1}), thus reducing the potential for NO_3^- leaching losses by half (Groves & Bailey, 1997). To date, few experiments have measured the effect of good irrigation management on NO_3^- leaching losses from cow urine patches deposited during the irrigation season, thus representing a gap in the knowledge.

Recently, there has been increased interest in deficit or water limited irrigation regimes and the utilisation of forage species or mixtures that are better suited to high temperatures or summer drought conditions (Neal *et al.*, 2009; Neal *et al.*, 2011; Minnee *et al.*, 2013; Nobilly *et al.*, 2013). Deficit irrigation implies less water or drainage below the root zone, however this also typically corresponds with a decrease in crop growth and N uptake, therefore the final effect on the amount of NO_3^- leached below the root zone remains unclear. The effect of deficit and optimum irrigation on herbage DM yield for a range of forage species was measured in an Australian trial by Neal *et al.* (2009). Although the results showed that deficit irrigation reduced the DM yield of all perennial forages, the study demonstrated the importance of forage choice under deficit irrigation regimes. For example, under deficit irrigation the annual DM yield for lucerne decreased by 22%, followed by chicory with a 38% decrease in DM yield. The least tolerant species was white clover which had a 74% decrease in annual DM yield. Variation in root architecture (i.e. the presence of tap roots) and the ability to adapt to drought were thought to be the key factors accountable for the large differences in DM yield between the forage species. However, more accurate information on the response of different forage species to water shortages is required to determine if alternative forage species or diverse forage mixtures could be used to reduce the risk of NO_3^- leaching from urine patches in water limited systems.

The type of irrigation system used can affect the quantity, intensity and timing of water application, which in turn can affect NO_3^- movement down the soil profile (Table 2.3). Once leached below the root zone, nutrients such as NO_3^- can no longer be utilised by the plants and are susceptible to leaching if drainage occurs. A *meta*-analysis by Quemada *et al.* (2013) found that management practices which adjust water application rates to meet crop needs, reduced NO_3^- leaching by 80% without reducing crop yield.

Table 2.3. Nitrogen leaching losses as affected by irrigation type.

Soil type	Crop type	N source	Rate (kg N ha^{-1})	Season applied	Method of irrigation	Application frequency (mm)	Mineral N leaching loss	Reference
Fine sandy loam	PRWC*	Dairy shed effluent	2 × 200	Summer and Autumn	Spray Flood	6 × 50 6 × 100	25.6 kg N ha yr^{-1} 13.1 kg N ha yr^{-1}	(Di *et al.*, 1998)
Fine sandy loam	PRWC	Ammonium fertiliser	2 × 200	Summer and Autumn	Spray Flood	6 × 50 6 × 100	49.0 kg N ha yr^{-1} 47.1 kg N ha yr^{-1}	(Di *et al.*, 1998)
Fine sandy loam	PRWC	Urine	1 × 1000	Autumn	Flood	6 × 100	124 kg NO_3^- ha yr^{-1}	(Silva *et al.*, 1999)
Fine sandy loam	PRWC	Urine Urea	1 × 1000 8 × 25	Spring	Spray Flood	16 × 25 8 × 100	192 kg N ha yr^{-1} 383 kg N ha yr^{-1}	(Moore, 2002)
Fine sandy loam	PRWC	Urine	1 × 1000	Autumn	Flood	6 × 100	77 kg NO_3^- ha yr^{-1}	(Di *et al.*, 2002)
Stony silt loam	PRWC	Urine Urea	1 × 1000 4 × 50	Spring	Flood	8 × 100	397 kg NO_3^- ha yr^{-1}	(Di & Cameron, 2002b)
Clay loam	Maize	Urea (fertigation)	4 × 37.5 4 × 37.5	Summer	Spray	1.0 SMD** 1.13 SMD	3.1 kg NO_3^- ha yr^{-1} 8.4 kg NO_3^- ha yr^{-1}	(Gheysari *et al.*, 2009)
Clay loam	Corn	Pig slurry Ammonium nitrate	1 × 365 1 × 125	Spring	Flood	1280 LE*** 860 HE	41.5 kg NO_3^- ha yr^{-1} 14.7 kg NO_3^- ha yr^{-1}	(Daudén *et al.*, 2004)
Sandy loam	Maize	Urea	1 × 460	Spring	Spray	1264 LE 1074 HE	166 kg N ha yr^{-1} 43 kg N ha yr^{-1}	(Diez *et al.*, 1997)

* Perennial ryegrass and white clover ** Soil moisture deficit *** Low and high irrigation efficiency

Flood irrigation in particular, often results in water being applied in excess of plant requirements or what can be held in the root zone of the soil. This can result in drainage and the potential for NO_3^- leaching losses to occur. For example, Daudén *et al.* (2004) reported the effects of high and low irrigation rates on NO_3^- leaching and found that over-irrigation of corn caused a significant increase in NO_3^- leaching from 14.7 kg NO_3^- ha yr^{-1} under low intensity irrigation to 41.5 kg NO_3^- ha yr^{-1} under high intensity irrigation. Low rate, high frequency irrigation was also shown to reduce the total amount and depth of NO_3^- leached from N fertiliser when applied to wheat (*Triticum aestivtcm* L.) sown in undisturbed columns (Bauder & Montgomery, 1980). In this greenhouse experiment, high volume, low frequency irrigation events leached NO_3^- to a greater depth than the low rate, high frequency irrigation treatment. Although in total both treatments received approximately the same amount of water, greater soil moisture depletion between irrigation events in the high rate, low frequency treatment resulted in greater plant stress and therefore lower DM yields. Similarly, Yahdjian and Sala (2010) reported greater NO_3^- leaching losses after a large irrigation event (pulse of 15 mm in 1 day) compared with smaller ones (three pulses of 5 mm each on three consecutive days) (Figure 2.4).

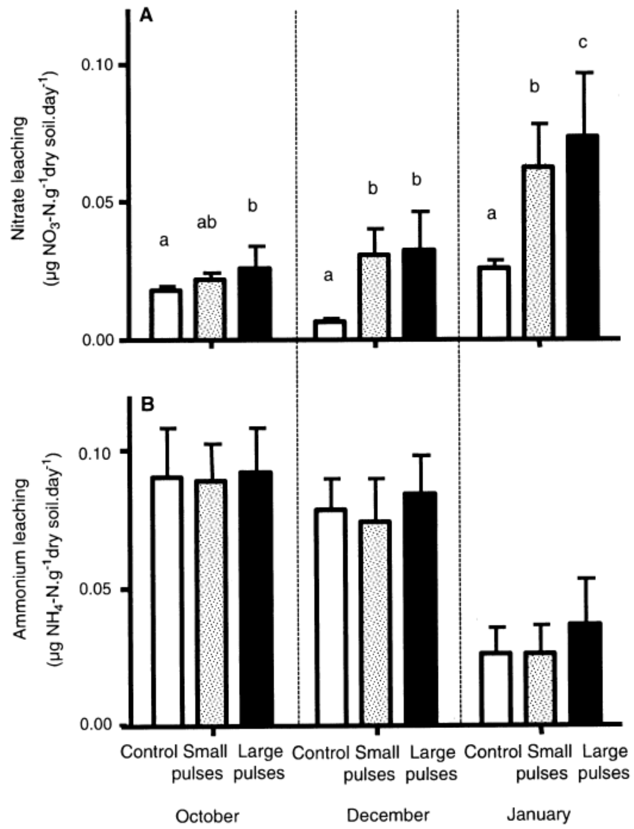


Figure 2.4. Nitrogen leaching from the upper soil in irrigation treatments and control. A Nitrate leaching; B ammonium leaching. Bars represent mean values (+SE) for n = 20 (Yahdjian & Sala, 2010).

To date, few studies have measured the effect of irrigation type on NO_3^- leaching losses from cow urine patches. Unpublished data from Moore (2002) reported the effect of flood and spray irrigation on NO_3^- leaching from spring deposited cow urine patches (1000 kg N ha^{-1}) and found that the flood irrigation caused a significant increase in NO_3^- leaching from 192 kg N ha yr^{-1} under spray irrigation to 383 kg N^- ha yr^{-1} under flood irrigation. However, in this experiment, the amount of water applied was twice as high for flood irrigation (100 mm every 3 weeks vs. 25 mm every 2 weeks). This resulted in a greater drainage flux that in turn leached more N than the spray irrigation treatment. While there was no difference in DM yield between the two irrigation treatments, water use efficiency was greater under the spray irrigation treatment. In contrast to this, Di *et al.* (1998) reported significantly less mineral N leaching from dairy shed effluent under flood irrigation (13.1 kg N ha^{-1}) when compared with spray irrigation (25.6 kg N ha^{-1}). However, while a greater amount of water was applied to the lysimeters under flood irrigation, the small N leaching losses were thought to have resulted from a greater amount of denitrification from the combined effects of wetter conditions and additional organic carbon.

Despite the increasing use of irrigation in New Zealand, it appears the literature contains little information relating to the effect of different irrigation types on NO_3^- leaching losses from cow urine patches deposited on perennial ryegrass and white clover forages. Furthermore, there appears to be no information relating to the performance of diverse forage species such as plantain and chicory under the different irrigation types used in New Zealand. This represents a gap in knowledge and an opportunity to better understand the effect of irrigation type on forage production and NO_3^- leaching losses from cow urine patches.

2.7 Conclusions

The main conclusions drawn from this literature review are:

- Considerable research has been carried out on NO_3^- leaching losses from autumn deposited urine patches however, few studies have measured NO_3^- leaching losses from early spring or summer deposited urine patches under irrigation.

- Earlier studies have indicated that diverse forages (i.e. containing the herbs plantain and chicory) may have the potential to occupy different soil niches and therefore increase DM production and N uptake.

- Lower urinary–N excretion has been measured from cows grazing diverse forages, particularly those containing plantain, compared with perennial ryegrass and white clover forages. It may therefore be possible that NO_3^- leaching losses could be reduced as a result of lower N loading in the urine patch. However, further research is required to make direct measurements of NO_3^- leaching losses from diverse vs. standard forages.

- Recent research has shown that forage species, such as plantain, contain secondary plant compounds that when released into the soil can inhibit nitrification. To date, there is no published data on nitrification inhibition by plantain in high N environments such as the urine patch. This represents a critical knowledge gap and a potential opportunity to mitigate NO_3^- leaching losses from cow urine patches.

- The area of agricultural land in New Zealand under irrigation is increasing. Despite this there are few studies that have quantified the effect of irrigation type on plant N uptake and subsequent NO_3^- leaching losses from cow urine patches. This is an area of research that requires further attention to better understand how irrigation can affect N cycling in grazed systems.

Chapter 3

General materials and methods

3.1 Introduction

Two lysimeter experiments were conducted on the Lincoln University Research Dairy Farm (LURDF) near Lincoln, Canterbury, New Zealand (43°38'S, 172°27'E) (Figure A.1). In Chapter Four, all lysimeters received a single urine application in November 2014, and measurements were taken thereafter. A new set of lysimeters were collected for Chapter Six, and a single urine application was applied to one set of lysimeters in December 2015 or on to another set of lysimeters in February 2016, and measurements as per Chapter Four were taken thereafter. A description of the site history, soil properties, lysimeter and urine collection, and field measurements is given below. The experimental design, and experimental methods and analysis are given in each chapter.

3.2 Site history

The lysimeters in Chapter Four were taken from adjacent irrigated paddocks sown in a standard ryegrass and white clover forage or a diverse forage (Figure A.1). From August to mid-May each year, both paddocks were rotationally grazed by Friesian × Jersey dairy cows. Six months prior to lysimeter collection, each site was fenced off to prevent cow urine patches from being deposited on the collection site.

The lysimeters in Chapter Six were taken from irrigated plots located 10 m apart which were sown in a standard or a diverse forage (Figure A.1). The plots were located near the lysimeter collection site for Chapter Four, and were mown periodically (c. monthly) to a residual height of 50 mm (c. 1500 kg DM ha^{-1}) prior to the lysimeters being collected. From the time the plots were sown the area was not grazed.

3.3 Soil properties

The soil used in both experiments was a well-drained Paparua fine sandy loam (Typic Immature Pallic soil (Hewitt, 2010); USDA: Udic Haplustept (Soil Survey Staff, 2014)), which consists of a fine sandy loam over a loamy sand. The parent material is alluvium derived from greywacke and the soil is described as moderately permeable with a high available water content (mm) in the root zone. The topsoil is stoneless and there are no significant root barriers within a depth of 1 m. The soil profile description is given in Table 3.1. In both experiments, soil cores (0-75 mm) were taken from each collection site and soil properties analysed (Table 3.2).

Table 3.1. Profile description of a Paparua soil located at the Lincoln University Research Dairy Farm (R. McLenaghen, personal communication, September 2014).

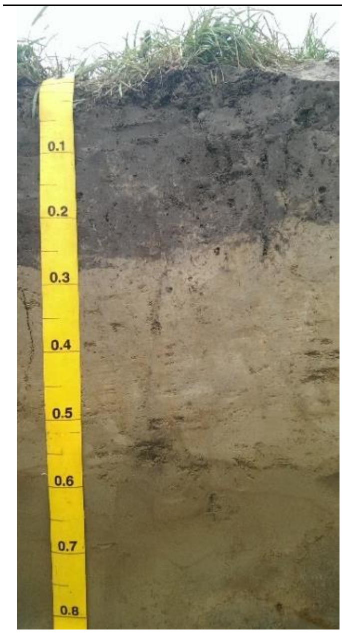
Soil profile	Horizon	Depth (cm)	Description
	Ah	0-25	Brownish black (10 YR 3/2); fine sandy loam; moderately weak; semi deformable; moderately developed nutty structure; abundant fine roots.
	Bw	25-55	Dull yellowish brown (10 YR 5/3); fine sandy loam; moderately weak; brittle; weakly developed nutty structure; few fine roots.
	BC	55-100	Greyish yellow brown (10 YR 5/2); loamy sand; loose; structureless single grained consistency; on stones.

Table 3.2. Soil properties (0-75 mm) from lysimeter collection sites sown in a standard or diverse forage for Experiments One and Two.

	Chapter Four		Chapters Five & Six	
Soil property	**Standard**	**Diverse**	**Standard**	**Diverse**
pH	5.6	5.6	6.9	6.8
Olsen P (μg mL^{-1})	28	26	17	20
Total N (% w / w)	-	-	0.21	0.24
Total C (% w / w)	-	-	1.8	2.62
Sulphate S (μg g^{-1})	17	6	2	5
CEC ($cmol_c$ kg^{-1})	-	-	16	15
Ca ($cmol_c$ kg^{-1})	10	9	18	13
Mg ($cmol_c$ kg^{-1})	12	12	22	12
K ($cmol_c$ kg^{-1})	8	5	7	4
Na ($cmol_c$ kg^{-1})	8	8	9	10

3.4 Lysimeter collection

Intact soil monolith lysimeters (500 mm diameter × 700 mm depth) were taken from the four collection sites at LURDF using established protocols (Cameron *et al.*, 1992). In brief, a cylindrical metal casing was placed on the soil surface and soil was dug out around it (Plate 3.1). The casing was gently pushed down in 150 mm increments to a depth of 680 mm. A circular wooden block (20 mm thick) was placed on the soil surface inside the lysimeter casing to ensure the lysimeter was collected to the desired depth (700 mm), and the soil surface was even with the lysimeter casing. A cutting plate was then used to separate the soil monolith from the underlying subsoil (Plate 3.1). The cutting plate was bolted to the metal casing. Liquefied petroleum jelly was carefully poured down the internal edge of the lysimeter to seal the gap between the casing and the soil monolith to reduce edge-flow effects. The lysimeter was then removed from the site for processing (Plate 3.1). To ensure uniform drainage, the bottom 50 mm of soil was replaced with gravel (representing drainage conditions at the site) (Plate 3.1). A circular drainage plate was then permanently attached to the base of the lysimeter and sealed with silicon sealant. Lysimeters were then installed in a trench facility located at LURDF (Plate 3.1). Tubing was attached to the drainage outlet in the base plate of each lysimeter allowing drainage to be collected in 10 L collection vessels. The lysimeters were then surrounded with soil to the same level as the lysimeter surface. This enabled the lysimeters to be exposed to the same climatic conditions as the surrounding field.

(a) Lysimeters collected in the field with and without 20 mm block of wood.

(b) Petroleum jelly to fill the gap between the casing and soil column.

(c) Hydraulic ram and cutting plate used to separate the lysimeter from the surrounding soil.

(d) Lysimeter being removed for processing.

(e) Upside down lysimeter with the bottom 50 mm of soil replaced with gravel.

(f) Lysimeters installed in trench facility.

Plate 3.1. Lysimeter collection, preparation and installation at LURDF (a) to (f).

3.5 Urine collection

For both experiments, fresh cow urine was collected during the afternoon milking on the Lincoln University Dairy Farm (LUDF) (Plate 3.2). The urine was collected from Friesian × Jersey dairy cows that had been grazing a perennial ryegrass and white clover forage prior to urine collection. Urine was immediately analysed for total N concentration using an ElementarVario-Max CN Elemental Analyser (Elementar, Gm, bH, Hanau, Germany), and standardised to the desired N concentration using urea and glycine in a 9:1 ratio (Bathurst, 1952). In Chapter Four all lysimeters received a 2 L surface application of cow urine, at a N loading rate of 500 or 700 kg N ha^{-1}, to replicate the urine patches deposited by grazing dairy cows (Selbie *et al.*, 2015) (Plate 3.2). In Chapter Six, lysimeters received a 2 L surface application of cow urine, at a N loading rate of 700 kg N ha^{-1}, either early or late summer. The control lysimeters did not receive urine, and 2 L of water was applied to maintain a similar soil moisture content across all treatments. Prior to the urine application, herbage was harvested to a residual height of 50 mm (c. 1500 kg DM ha^{-1}).

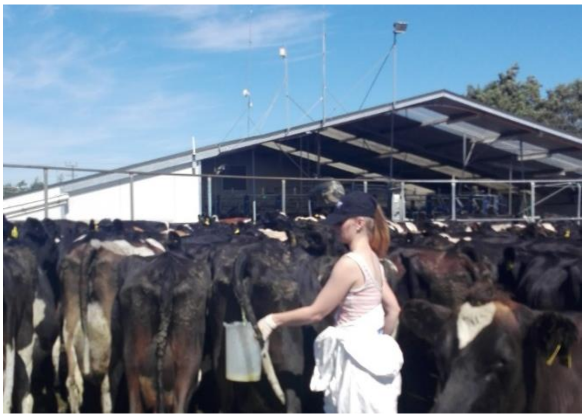



Plate 3.2. Urine collection from dairy cows during afternoon milking (left), and the application of cow urine onto the lysimeter (right).

3.6 Field measurements

3.6.1 Leachate

Leachate was collected in 10 L containers which were connected to the base of each lysimeter and housed in a metal box (Plate 3.3). Leachate from the lysimeters was collected following a drainage event, which was at least weekly during the winter period. Two 50 mL sub-samples were collected from each lysimeter and frozen prior to chemical analysis, and total drainage volumes were recorded for each lysimeter (Plate 3.3).

Plate 3.3. Leachate collection container attached to the base of the lysimeter (left), and 50 mL samples collected for chemical analysis (right).

3.6.2 Herbage

Herbage was harvested once plant development had reached the two–three leaf stage when yields were on average 3000 kg DM ha^{-1}. Herbage was cut using electric hand shears to a residual height of 50 mm (c. 1500 kg DM ha^{-1}) (Plate 3.4). All harvested material was removed and oven-dried at 60 °C for 48 hours, and dry matter (DM) production was determined. A sub-sample was finely ground and analysed for total N concentration using an Elementar Vario-Max CN Elementar Analyser (Elementar GmbH, Hanau, Germany) (Plate 3.4).

In Chapter Four, a botanical composition was determined for each lysimeter from an autumn harvest. This involved separating individual forage species into bags and obtaining a dry weight for each to determine the percentage of each species in the forage mixture (Tothill *et al.*, 1992). Samples were then bulked by lysimeter and finely ground for chemical analysis as per the other harvests.

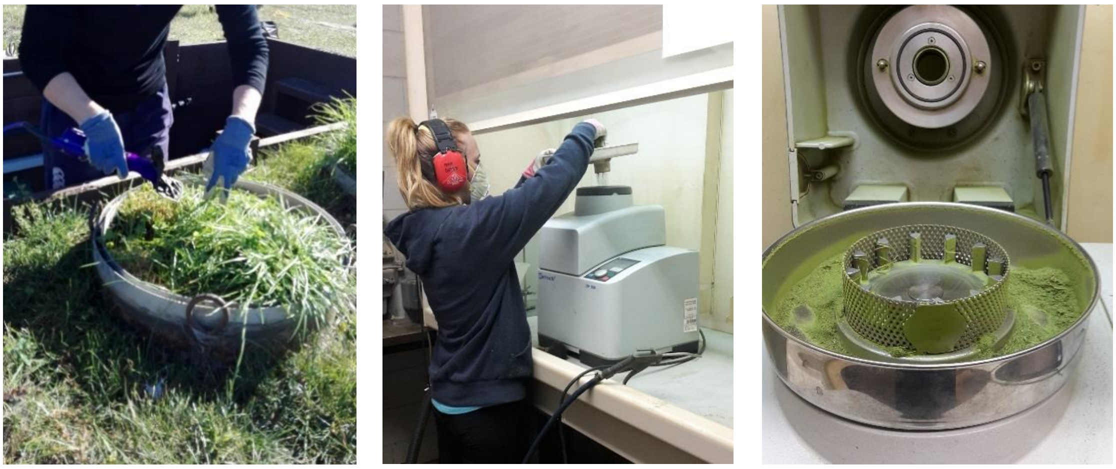


Plate 3.4. Herbage was cut using electric hand shears (left), and then finely ground for analysis (centre and right).

3.6.3 Soil moisture

Soil moisture sensors (Campbell Scientific Water Content Reflectometers, CS615) were installed in one lysimeter from each treatment to monitor the soil moisture content at a range of depths. In Chapter Four, three sensors were installed diagonally (41.8° off horizontal) in the selected lysimeters at depths of 0–200 mm, 200–400 mm and 400–600 mm. In Chapter Six, two sensors were installed diagonally (41.8° off horizontal) in each of the selected lysimeters at depths of 0–300 mm and 200–400 mm. The sensors measured volumetric soil content, which was used to adjust irrigation application when necessary.

The top moisture sensor (depth 0–200 mm) was installed by gently pushing the moisture sensor into the soil on top of the lysimeter (Plate 3.5). To install the middle and bottom sensors, two holes were drilled into the lysimeter casing, and the sensor installed as shown below (Plate 3.5). Silicone was then applied around the base of the probe creating a water tight seal, and soil was backfilled around the outside of the lysimeter.

The soil moisture sensors were wired to a CR 1000 Campbell Scientific data logger, which was set up to determine soil moisture data every 10 minutes, 24 hours per day.

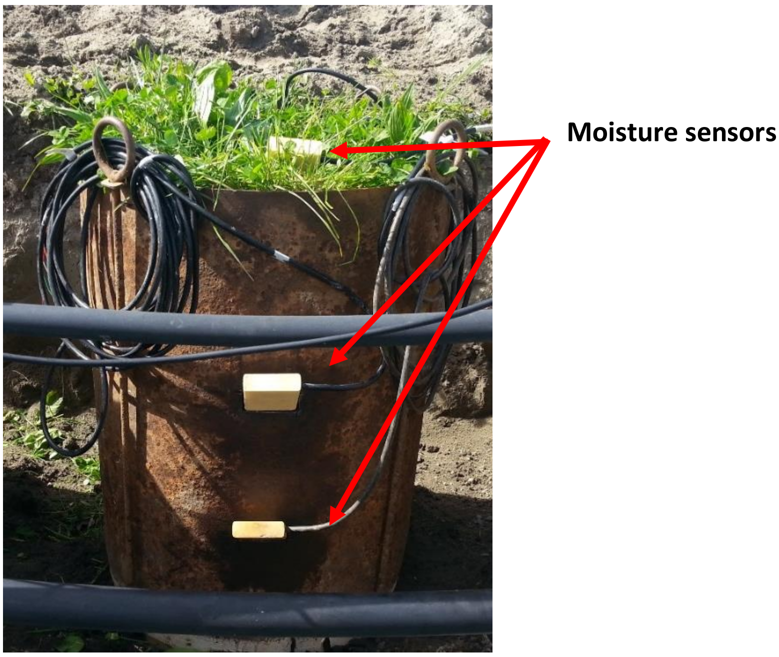


Plate 3.5. Soil moisture sensors installed in the lysimeter at depths of 0–200 mm, 200–400 mm and 400–600 mm.

3.6.4 Soil temperature

Temperature sensors (Campbell Scientific Temperature Sensors, 107) were installed in two lysimeters in Chapter Four at depths of 100 mm and 300 mm, and three lysimeters in Chapter Six at a depth of 100 mm. To install the temperature sensor, a single hole was drilled into the lysimeter casing and the temperature probe inserted into the soil. Silicone was then applied around the base of the sensor creating a water tight seal. Measurements were recorded as described for the soil moisture probes.

3.7 Rainfall and irrigation simulation model

Water applications for both experiments were set to simulate actual rainfall and irrigation events. The system was primarily controlled by historical and daily climate data (NIWA Lincoln Broadfield Weather Station, Canterbury, New Zealand), and was driven by a CR 1000 Campbell Scientific data logger. Additional daily climate data including rainfall, wind speed, solar radiation, and air and ground temperatures were obtained from a weather station located at the field site. A full description of the rainfall and irrigation system can be found in Malcolm (2013).

In brief, during rainfall simulation mode (April to September) natural rainfall was supplemented with simulated rainfall, when necessary, to maintain water inputs at the 75th percentile of the regional average between 1975 and 1998/99. This ensured sufficient drainage was generated during the winter/spring period, and a full nitrate breakthrough curve was obtained. During the irrigation simulation mode (October to March) the amount, frequency and intensity of each water application was user defined. However, when natural rainfall occurred, the period of time between the irrigation events was extended to remain consistent with typical irrigation practice and daily evapotranspiration rates. The irrigation water used was sourced from an underground water supply with NO_3^-–N concentrations below 1 mg N L^{-1}.

Water was applied to individual lysimeters through Tee Jet FL-5VC spray nozzles mounted directly over the top of each lysimeter (Plate 3.6), with the exception of flood irrigated lysimeters in Chapter Six where irrigation water was manually applied. The system was calibrated to apply water at a rate of 1000 mL per minute in 0.5 mm bursts for pivot and rotorainer irrigation, and simulated rainfall.

Plate 3.6. Sprinkler system applying water to the lysimeters.

3.8 Statistical analysis

Statistical analysis in Chapters Four, Five and Six were carried out using analysis of variance (ANOVA) using Genstat (16th Edition, VSN International Ltd). Standard errors of the mean were calculated and presented with the mean values. Normality was checked by examining a histogram of the residuals from the fitted model, and by examining a Q-Q plot of the model residuals against the quantiles of the normal distribution. Homogeneity of variance was checked by plotting the model residuals against the fitted model values. Where necessary, data were log-transformed to normalise the variance and then determine statistical treatment effects.

Chapter 4

Effect of irrigation management, forage type and urinary nitrogen loading rate on nitrate leaching losses

4.1 Introduction

Urinary nitrogen (N) deposited by grazing dairy cows presents a significant environmental problem in New Zealand because nitrate (NO_3^-) derived from this urine contributes to surface and ground water contamination (Cameron *et al.*, 2013). A mismatch between animal metabolic requirements and the N composition of New Zealand forages can result in 60–90% of N consumed being deposited back onto the paddock, 70% of which is deposited as urine (Di & Cameron, 2002a). Typically, N deposited in the urine patch exceeds plant nutritional requirements thus surplus N (once in the form of NO_3^-) can be leached from the soil profile when drainage occurs (Cameron *et al.*, 2013). Under the New Zealand National Policy Statement for Freshwater Management, regional councils are currently developing regulations that place a limit on the amount of NO_3^- loss from individual farms (Ministry for the Environment, 2014). This may require substantial changes in typical farm practices to reduce NO_3^- loss to below current levels, and a resource consent may need to be obtained to continue an existing land use (Chapman *et al.*, 2014; Ministry for the Environment, 2014). The development of mitigation strategies to reduce NO_3^- loss on a large scale in New Zealand are therefore critical to meeting environmental and regulatory obligations.

4.1.1 Forage type

Recently there has been increased interest in the role of diverse forages (e.g. containing plantain [*Plantago lanceolata*] and chicory [*Cichorium intybus*]) to overcome some of the limitations of standard perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) based grazing systems (Pembleton *et al.*, 2015; Box *et al.*, 2016; Bryant *et al.*, 2017). Of key interest is how these diverse forages respond to irrigation and whether they can be used to reduce NO_3^- leaching losses. Previous irrigation studies have shown that diverse forages can produce greater dry matter (DM) yields compared to standard forages (Goh & Bruce, 2005; Nobilly *et al.*, 2013). Nobilly *et al.* (2013) reported that with the inclusion of plantain, chicory, and legumes, red clover (*Trifolium pratense*) and lucerne (*Medicago sativa*), DM production was greater from diverse than standard forages by 1.62 t DM ha^{-1} (averaged over 2 years). Furthermore, through niche separation, diverse forages may be better suited to dryland or water-limited irrigation systems (deficit irrigation) due to deeper rooting depth and the ability to extract water and nutrients from deeper soil layers (Skinner *et al.*, 2004; Sanderson *et al.*, 2007).

Hypothesis: Under 'optimum' and 'deficit' irrigation, herbage DM yield and N uptake will be greater by diverse forages due to the presence of the deep rooting species plantain and chicory.

4.1.2 Irrigation

Research in cropping has shown that NO_3^- leaching can be reduced under optimum irrigation compared to dryland systems (Hahne *et al.*, 1977; Groves & Bailey, 1997; Paramasivam *et al.*, 2001; Gheysari *et al.*, 2009). For example, Groves and Bailey (1997) found that optimum irrigation reduced residual soil N by 31 kg N ha^{-1} compared with the unirrigated treatment (79 kg N ha^{-1}) and thus the potential for NO_3^-–N leaching during winter was halved. However, few studies have investigated NO_3^- leaching losses from the urine patch under optimum and deficit irrigation regimes.

Hypothesis: Optimum irrigation will increase herbage N uptake and therefore reduce NO_3^- leaching losses from spring deposited urine compared with deficit irrigation.

4.1.3 Urinary-N excretion

In addition, diverse forages containing plantain and chicory have been reported to reduce urinary–N excretion from dairy cows, without compromising milk production (Table 4.1). Edwards *et al.* (2015) reported a 20% decrease in urinary–N excretion from cows grazing a diverse forage compared to a perennial ryegrass and white clover forage. Milk production, urination frequency and volume were reported to be similar for both forages, thus demonstrating the potential to reduce NO_3^- leaching losses through reduced N loading in the urine patch.

Table 4.1. Urinary–N excretion from dairy cows grazing on a standard perennial ryegrass and white clover forage vs. diverse forages.

Reference	Forage type	Plant species in forage	Urinary–N excretion
(Woodward *et al.*, 2012)	Standard	RGWC*	200 g N day^{-1}
	Diverse	RGWC, plantain, chicory and lucerne	100 g N day^{-1}
(Totty *et al.*, 2013)	Standard	RGWC	438 g N day^{-1}
	Diverse	RGWC, plantain, chicory and lotus	354 g N day^{-1}
(Edwards *et al.*, 2015)	Standard	RGWC	6.1 g N L^{-1}
	Diverse	RGWC, plantain, chicory and lucerne	4.9 g N L^{-1}
(Box *et al.*, 2016)	Standard	RGWC	5.4 g N L^{-1}
	Diverse	50:50 RGWC and plantain	3.6 g N L^{-1}

* Perennial ryegrass and white clover

Hypothesis: Nitrate leaching losses will be lower from urine deposited by cows grazing a diverse forage compared with cows grazing a standard forage containing perennial ryegrass and white clover.

4.1.4 Objectives

Currently, few studies have measured the effects of irrigation management <u>and</u> N loading rate on NO_3^- leaching losses and N uptake of diverse and standard forages. The objective of this experiment was therefore to quantify the effect of optimum vs. deficit irrigation management regimes on N uptake by diverse and standard forages, and the subsequent effects of these irrigation regimes on NO_3^- leaching losses from spring deposited urine.

4.2 Materials and methods

4.2.1 Experimental design and treatments

The experimental design consisted of eight treatments including two urinary–N application rates (500 vs. 700 kg N ha^{-1}), two forage types ('standard' vs. 'diverse') and two irrigation regimes ('deficit' vs. 'optimum') (Table 4.2). Treatments were arranged in a randomised block design and replicated five times. Dairy cow urine was applied on the 19 November 2014 and measurements were taken thereafter until the 30 September 2015. Prior to treatment application, water was applied to all lysimeters to flush any pre-existing NO_3^-–N from the soil. Soil moisture probes were used to ensure the soil in the lysimeters was fully saturated.

Table 4.2. Description of lysimeter treatments.

Treatment no.	Irrigation regime	Irrigation rate (mm) and frequency (days)	Forage species	Urine treatment (kg N ha^{-1})
1	Deficit	9 mm every 3 days	Standard	700
2	Deficit	9 mm every 3 days	Diverse	700
3	Deficit	9 mm every 3 days	Standard	500
4	Deficit	9 mm every 3 days	Diverse	500
5	Optimum	18 mm every 3 days	Standard	700
6	Optimum	18 mm every 3 days	Diverse	700
7	Optimum	18 mm every 3 days	Standard	500
8	Optimum	18 mm every 3 days	Diverse	500

4.2.2 Urine collection and application

On the 18 November 2014 (late spring), fresh urine was collected from Friesian × Jersey cross cows on the Lincoln University Dairy Farm (LUDF) during afternoon the milking. Prior to urine collection the cows had been grazing a perennial ryegrass and white clover forage. The total urinary–N concentration was 4.49 g N L^{-1}, this was standardised to concentrations of 5 g N L^{-1} or 7 g N L^{-1} using ^{15}N enriched and natural abundance urea, and glycine in a 9:1 ratio (Bathurst, 1952). The isotopic ^{15}N label was added to the urine to determine the contribution of urinary–N to NO_3^-–N leaching. Highly enriched ^{15}N urea (98 atom%) was used to give a ^{15}N abundance of 5 atom%. Individual lysimeters received a 2 L surface application of urine to simulate typical urine patch deposition by grazing dairy cows, at a loading rate of 500 or 700 kg N ha^{-1}. Urine patch N loading rates are variable, however recent literature has shown that typical deposition rates from cows grazing on diverse forages are around 500 kg N ha^{-1}, and are around 700 kg N ha^{-1} from cows grazing on a standard perennial ryegrass and white clover forage (Totty *et al.*, 2013; Selbie *et al.*, 2015).

4.2.3 Forage type

The forages from both collection sites were sown in spring 2011 and at the time of lysimeter extraction were three years old. The standard forage contained perennial ryegrass and white clover. The diverse forage contained perennial ryegrass, white clover, red clover, prairie grass, chicory and plantain. Sowing rates are given in Table 4.3.

Herbage was harvested once plant development had reached the two–three leaf stage, or under optimum irrigation when yields were on average 3000 kg DM ha^{-1}. Harvests were managed according to plant growth for the two irrigation treatments and were uniform across the two forage types.

Table 4.3. Species, sowing rate and cultivar for standard and diverse forage types.

			Sowing rate (kg seeds ha^{-1})	
Plant species	**Scientific name**	**Cultivar**	**Standard forage**	**Diverse forage**
Perennial ryegrass	*Lolium perenne* L.	Expo AR1 Endophyte	20	10
White clover	*Trifolium repens* L.	Weka	3	3
Red cover	*Trifolium pratense* L.	Colenso		5
Prairie grass	*Bromus willdenowii* L.	Atom		15
Chicory	*Cichorium intybus* L.	Choice		1.5
Plantain	*Plantago lanceolata* L.	Tonic		1.5

4.2.4 Irrigation scheduling

From November to April (summer) two spray irrigation regimes were simulated (Table 4.4). "Optimum" irrigation comprised of irrigation events every three days, with applications of 18 mm at an intensity of 20 mm per hour. For the period 21 January 2015 to 28 February 2015, the optimum irrigation regime was increased to 24 mm because of the high evapotranspiration creating a large soil moisture deficit. Optimum irrigation was applied to match typical centre pivot irrigation rates in the Canterbury region (Hydroservices Ltd, 2014). “Deficit” irrigation was applied at c. 50% of the optimum irrigation regime and comprised of irrigation events every three days, with applications of 9 mm and at an intensity of 20 mm per hour. Deficit irrigation was applied at a rate that was representative of drought conditions. In the event of natural rainfall, the period of time between irrigation events was extended to remain consistent with typical irrigation practice. Water was applied to individual lysimeters at midnight to limit water loss from evapotranspiration and wind interference. The soil moisture sensors described in Section 3.6.3 were used to monitor the soil moisture content and adjust irrigation applications when necessary.

Table 4.4. Description of deficit and optimum irrigation regimes.

Irrigation treatment	Amount (mm)	Frequency (days)	Intensity (mm hr^{-1})	Duration
Optimum	18-24	3	20	Nov-Apr
Deficit	9	3	20	Nov-Apr

4.2.5 Fertiliser

Prior to treatment application, all lysimeters received a maintenance fertiliser application in the form of 20% potash super sulphur (6.4–10–16–14). These were equivalent to 60 kg P ha^{-1} (diverse forage site) and 50 kg P ha^{-1} (standard forage site). Maintenance fertiliser was calculated using the soil test results in Table 3.2. To raise the soil pH, all lysimeters also received a lime application in the form of Ag lime, equivalent to 4 t lime ha^{-1}. Urea fertiliser was applied in split applications to provide an annual rate of 150 kg N ha^{-1}. Under optimum irrigation, as per recommended farm practice, fertiliser was applied between October and April in split applications of 25 kg N ha^{-1} (Fertiliser Assosiation, 2009). Under deficit irrigation, fertiliser was applied when there was sufficient soil moisture for plant growth in split applications of 25-35 kg N ha^{-1}. Nitrogen fertiliser was applied at the same annual rate for both irrigation treatments to avoid bias. All fertiliser was hand applied evenly across the surface of the lysimeter. This was followed with 10 mm of irrigation to wash the fertiliser into the soil and to prevent volatilisation.

4.2.6 Analysis

Leachate and herbage sub-samples were collected from individual lysimeters for laboratory analysis. Sampling methods are outlined in Section 3.6.

4.2.6.1 Leachate

Nitrate and ammonium

Leachate samples were analysed for NO_3^-–N and NH_4^+–N concentrations by flow injection analysis (FIA) using a FOSS FIAstar 5000 twin channel analyser (Foss Tecator AB, Hoganas, Sweden) (Gal *et al.*, 2004). Nitrate–N was analysed by the reduction of NO_3^-–N to NO_2^-–N using a packed cadmium reduction coil, followed by the reaction of NO_2^-–N with sulphanilamide/NED to form an azo dye compound. The intensity of the compound was then determined spectrophotometrically at 540 nm. Ammonium–N was determined using a gas diffusion membrane. Ammonium–N ions present in the leachate were converted to NH_3 gas by adding sodium hydroxide to the sample stream. The gas then diffused through the membrane into an indicator stream which changed colour (red to blue) with an increase at 590 nm. The extent of the colour change was then used to determine the concentration of NH_4^+–N ions present in the leachate (the method was adapted from FOSS Application Notes: AN 5206, AN 5232, AN 5226, AN 5220 and AN 5222).

^{15}N recovery

Due to low NO_3^-–N and NH_4^+–N concentrations (determined by FIA) in the leachates, only two treatments (deficit diverse 700 and deficit standard 700) were selected for ^{15}N analysis. Three replicates where chosen at random from each treatment, and samples with a NO_3^-–N concentration greater than 0.5 mg L^{-1} were diffused in preparation for ^{15}N analysis using a stable isotope ratio mass spectrometer (Sercon Ltd, Crewe, CWI6ZA, UK). For samples with NO_3^-–N concentrations that were too low for detection by the mass spectrometer, no diffusion or ^{15}N analyses was undertaken. This resulted in some treatments having fewer than three replicates. To reduce error and prevent over estimating it was assumed that these samples would have a ^{15}N recovered enrichment of zero due to the low NO_3^-–N concentration.

Following procedures outlined in Brooks *et al.* (1989), a pre-determined volume of sample was pipetted into a 120 mL plastic container, and where required deionised water was added to make a final volume of 50 mL. Two acid wash glass beads, 0.1 mL of 30% Brij-35, 0.2 g of MgO and 0.4 g of Devarda's alloy were added to the sample. Following this, 10 µL of 2.5 M $KHSO_4$ was added to a 7 mm diameter disc of Whatman GF/D filter paper which was suspended, using a wire, over the sample. The container was then closed and gently mixed. The containers were then left at room temperature for six days without further mixing. The discs were then carefully removed and dried in desiccator overnight before being placed into tin capsules and tightly folded.

The diffused samples were then analysed to determine the ^{15}N content using a stable isotope ratio mass spectrometer. Each sample was combusted at 1000°C in an oxygen atmosphere using an automated Dumas style elemental analyser converting N species to N_2 gas. The analyser was linked to a 20–22 stable isotope ratio mass spectrometer allowing the measurement of stable isotopes at both enriched and natural abundance levels.

The percentage recovery of ^{15}N in leachate and herbage was calculated using Equation 6 adapted from Cabrera and Kissel (1989):

$$\%^{15}N\ recovery = \frac{P\ (C-B)}{F\ (A-B)} \times 100 \qquad (6)$$

where:

P = moles of N in the measured fraction

C = atom % ^{15}N enrichment of the measured fraction

B = atom % ^{15}N natural abundance enrichment, which was 0.6071% (leachate) and 0.3663% (herbage)

F = moles of N in the urine applied to the lysimeter which was 0.7001 mol (500 kg N ha^{-1}) and 0.9812 (700 kg N ha^{-1})

A = atom % ^{15}N abundance of the urine applied to the lysimeter, which was 5 atom %

4.2.6.2 Herbage

Total nitrogen

Herbage was analysed for total N and carbon (C) concentration using an Elementar Vario-Max CN Elementar Analyser (Elementar GmbH, Hanau, Germany). Each sample was combusted at 900°C in an oxygen atmosphere. The combustion process converted any elemental C and N into CO_2, N_2 and NO_x. Any NO_x was subsequently reduced to N_2, and the gasses were then passed through a thermal conductivity cell to determine the total amount of CO_2 and N_2.

^{15}N recovery

Herbage was analysed for ^{15}N content using the stable isotope ratio mass spectrometer (Sercon Ltd, Crewe, CWI6ZA, UK). Each sample was combusted at 1000°C in an oxygen atmosphere in an automated Dumas style elemental analyser converting N species to N_2 gas. The analyser was linked to a 20-22 stable isotope ration mass spectrometer allowing the measurement of stable isotopes at both enriched and natural abundance levels. The percentage of ^{15}N in the herbage was calculated using Equation 6 in Section 4.2.6.1, adapted from Cabrera and Kissel (1989).

4.2.7 Statistical analysis

Statistical analysis was carried out using analysis of variance (ANOVA) (Genstat 16th Edition, VSN International Ltd). Standard errors of the mean were calculated and presented with the mean values. The total NO_3^- leached, herbage DM yield and N uptake, and ^{15}N recovery data were log-transformed to normalise the variance and to determine statistical treatment effects.

4.3 Results

4.3.1 Temperature

Daily air and ground temperatures are given in Figure 4.1. Minimum air and ground temperature was recorded on 2 July 2015 at 0.5°C and 1.4°C, respectively. Maximum air and ground temperature was measured on 18 February 2015 at 24°C and 23°C, respectively.

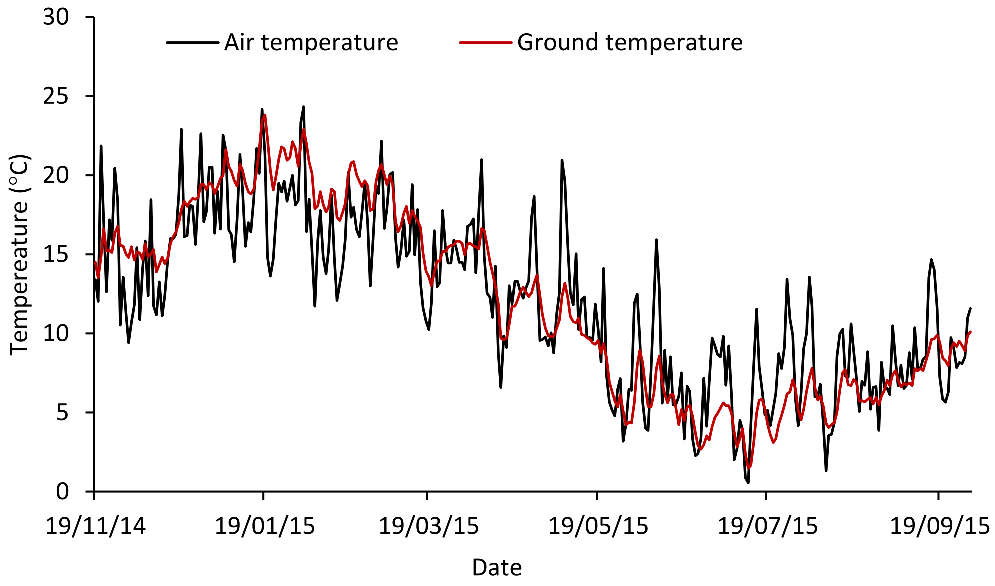


Figure 4.1. Average daily air and ground temperature from November 2014 to September 2015.

4.3.2 Water inputs, drainage and soil moisture

From November 2014 to April 2015, the total amount of irrigation applied was 716 mm under the optimum irrigation treatment, and 232 mm for the deficit irrigation treatment. Total rainfall for the experimental period was 644 mm, the majority of this occurred between July and September 2015 (Figure 4.2).

The greater water input under optimum irrigation resulted in a greater amount of drainage compared with deficit irrigation (Figure 4.3). The greatest amount of drainage was recorded during the winter and early spring period (July to September). Little drainage occurred over the summer period. Irrigation treatments had a significant effect ($P < 0.01$) on the total amount of drainage for the duration of the experiment (Figure 4.4). The greatest amount of drainage occurred under the standard forage treatments receiving optimum irrigation. Under the optimum irrigation treatment the amount of drainage was significantly greater ($P < 0.05$) from the standard forages than the diverse forage treatments (at both N loading rates).

During the irrigation season (November 2014 to April 2015), the optimum and deficit irrigation regimes produced different soil moisture contents in the upper depth of the lysimeters (0–200 mm). From May 2015 onwards, the soil moisture content followed a similar trend for both irrigation regimes (Figure 4.5).

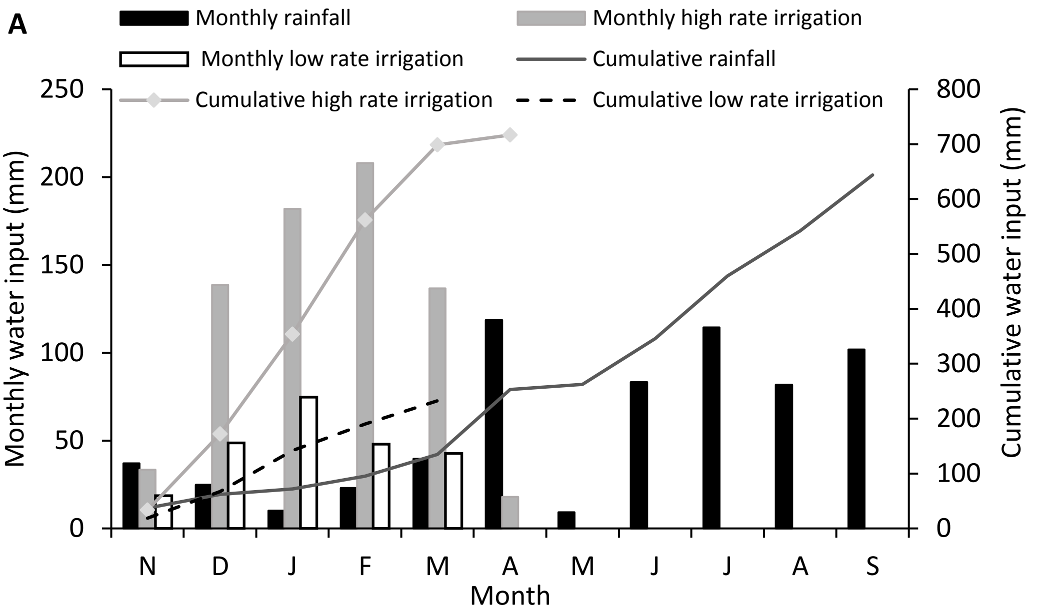


Figure 4.2. Cumulative and monthly water inputs (mm) from November 2014 to September 2015.

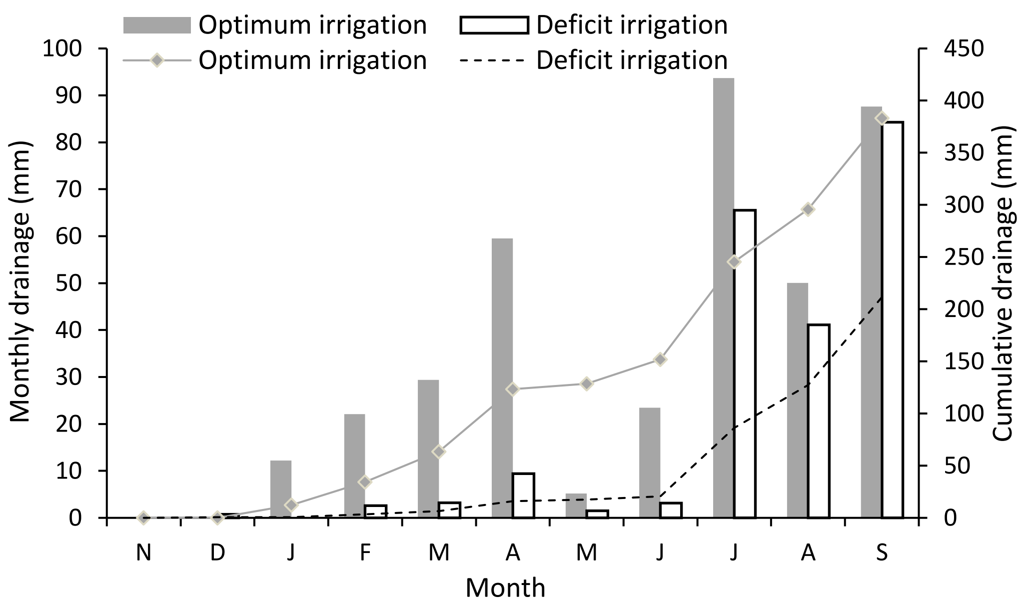


Figure 4.3. Cumulative and monthly drainage (mm) from November 2014 to September 2015.

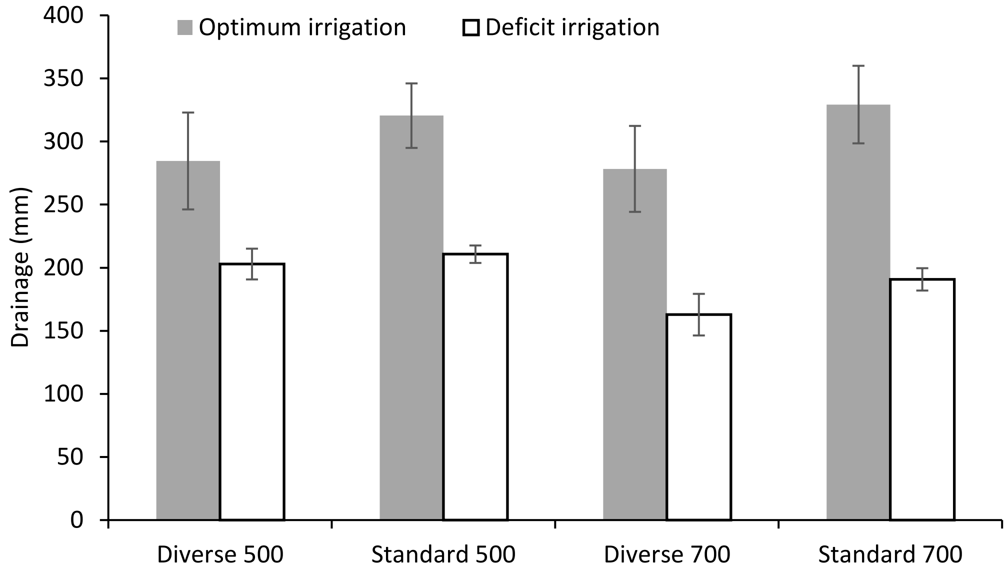


Figure 4.4. Total amount of drainage (mm) averaged from each treatment. Error bars are ± standard error of the mean (SEM). Number of replicates = 5.

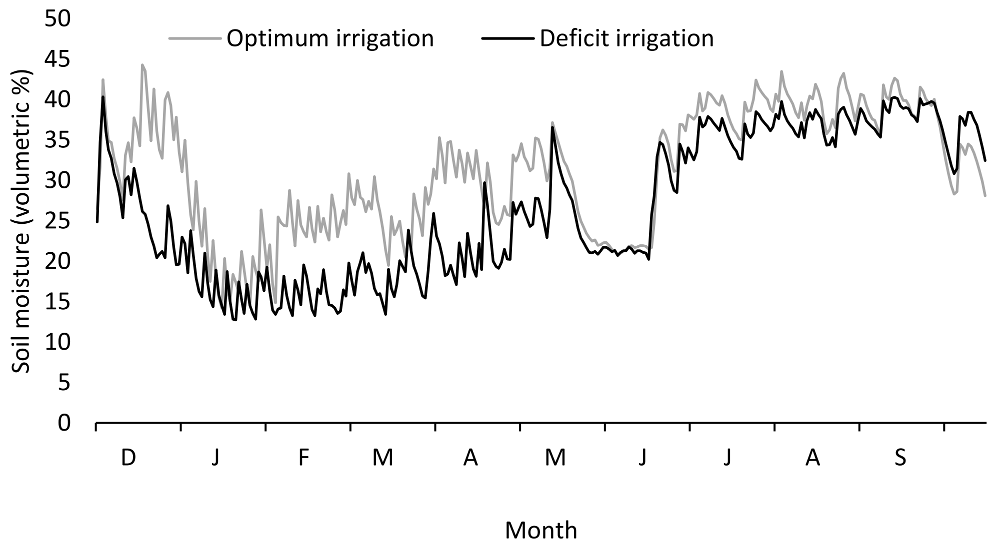


Figure 4.5. Soil moisture (volumetric %) at 0–200 mm for lysimeters receiving optimum or deficit irrigation.

4.3.3 Nitrate leaching losses

The form of N leached was predominantly NO_3^-–N with little or no NH_4^+–N detected in the leachate. The highest NO_3^-–N concentration in drainage water occurred under the deficit irrigation treatment with 27 mg NO_3^-–N L^{-1} recorded from the 700 kg N ha^{-1} urine treatment (Figure 4.6). The peak NO_3^-–N concentrations in drainage water under the optimum irrigation treatment remained below 1.7 mg NO_3^-–N L^{-1} for both rates of urine and both forage types (Figure 4.6).

Irrigation treatments had a significant ($P < 0.01$) effect on the total amount of NO_3^-–N leached from the soil (Table 4.5). The greatest amount of NO_3^-–N was leached under the deficit irrigation treatment when the urinary–N loading rate was 700 kg N ha^{-1} with 10 and 20 kg NO_3^-–N ha^{-1} leached from the standard and diverse forages, respectively (Figure 4.7). Nitrate leaching losses under the optimum irrigation were significantly ($P < 0.05$) less than from the deficit irrigation treatment. The NO_3^-–N leaching loss under optimum irrigation was 88% less than that under the deficit irrigation of the diverse forage that received the 700 kg N ha^{-1} urine treatment. The NO_3^-–N leaching loss under optimum irrigation was 97% less than that under deficit irrigation of the standard forage that received 700 kg N ha^{-1} (Figure 4.7). Leaching losses from the 500 kg N ha^{-1} urine treatment were below 4 kg N ha^{-1} for both forage types and there was no significant difference between the irrigation treatments. There was a trend towards NO_3^-–N leaching losses from diverse forage treatments with a loading rate of 500 kg N ha^{-1} being lower than those from the standard forage at a loading rate of 700 kg N ha^{-1} (representative of reported N loading rates for cows grazing diverse and standard forages) however, the reduction in NO_3^-–N leaching was not statistically significant ($P = 0.114$).

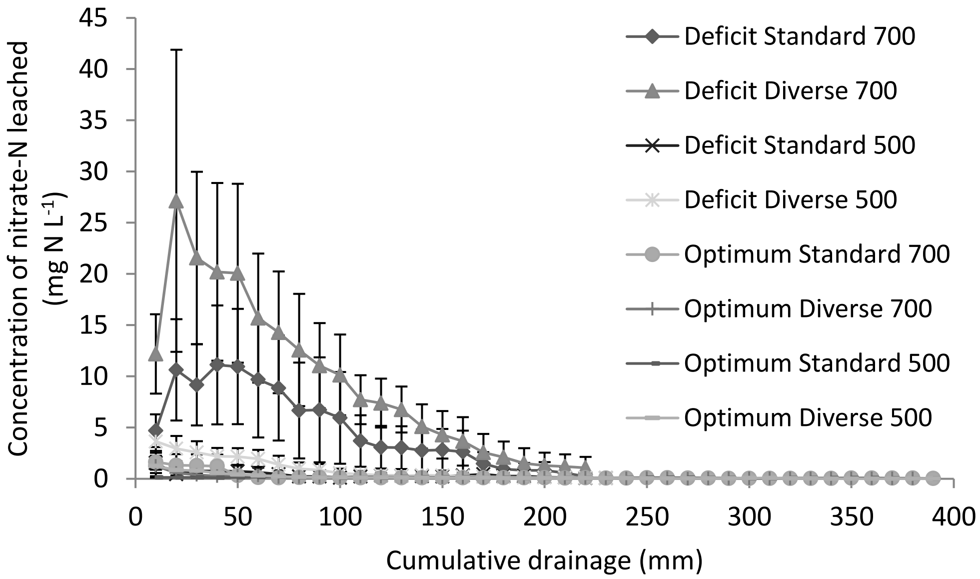


Figure 4.6. Concentration of NO_3^--N (mg L^{-1}) in drainage water as affected by irrigation (optimum vs. deficit), plant species (standard vs. diverse) and urine patch loading rate (500 vs. 700 kg N ha^{-1}). Number of replicates = 5.

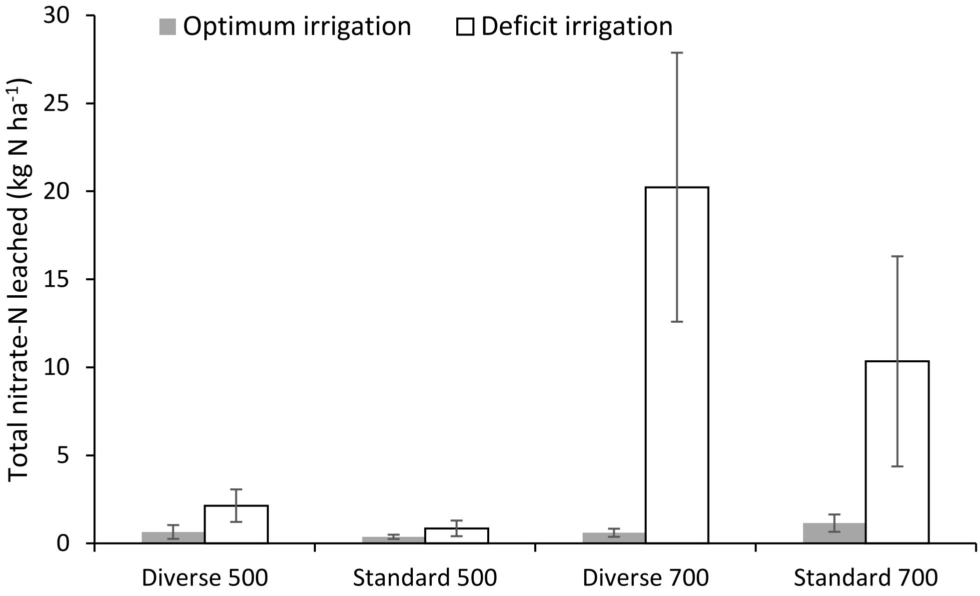


Figure 4.7. Total NO_3^-–N leached (kg ha^{-1}) from lysimeters as affected by irrigation (optimum vs. deficit), plant species (standard vs. diverse) and urine patch loading rate (500 vs. 700 kg N ha^{-1}). Error bars are ± standard error of the mean (SEM). Number of replicates = 5.

Table 4.5. Total nitrate leaching loss (kg NO_3^-–N ha^{-1}), herbage DM yield (kg DM ha^{-1}) and herbage N uptake (kg N ha^{-1}) from lysimeters during the experimental period.

			Log$_{10}$ means		
Irrigation	**Forage**	**Urine rate**	**Total NO_3^- loss (kg NO_3^-–N ha^{-1})**	**DM yield (kg DM ha^{-1})**	**N uptake (kg N ha^{-1})**
Deficit	Diverse	500	0.181	4.059	2.555
	Standard	500	-0.270	4.025	2.523
	Diverse	700	1.165	4.128	2.662
	Standard	700	0.639	4.101	2.631
Optimum	Diverse	500	-0.420	4.273	2.761
	Standard	500	-0.508	4.236	2.715
	Diverse	700	-0.312	4.269	2.773
	Standard	700	-0.147	4.258	2.746
LSD (5%) within irrigation regimes			0.566	0.040	0.044
LSD (5%) for all other comparisons			0.586	0.060	0.071
Significance of main effect					
Irrigation			**	***	***
Forage			NS	**	**
Urine rate			***	***	***
Significance of interaction					
Irrigation × forage			NS	NS	NS
Irrigation × urine rate			*	**	***
Forage × urine rate			NS	NS	NS
Irrigation × forage × urine rate			NS	NS	NS

NS = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

4.3.4 Herbage DM yield and N uptake

Averaged across all treatments, total herbage DM yield (t DM ha^{-1}) and N uptake (kg N ha^{-1}) were affected by irrigation, forage type and N loading rate with a significant interaction occurring between irrigation type and N loading rate (Table 4.5). Total herbage DM yield and N uptake under deficit irrigation was lower than under optimum irrigation and ranged from 10.6–13.5 t DM ha^{-1} and 334–460 kg N ha^{-1} (deficit irrigation) compared to 17.4–19.0 t DM ha^{-1} and 530–596 kg N ha^{-1} (optimum irrigation), respectively (Figure 4.8 and Figure 4.9). This represented a significant ($P < 0.05$) increase in herbage DM yield of 38–66% under optimum irrigation and importantly a 30–64% increase in herbage N uptake. Under deficit irrigation, herbage DM yield and N uptake were significantly ($P < 0.05$) greater from urine deposited at an loading rate of 700 kg N ha^{-1} compared to the 500 kg N ha^{-1} urine application rate and this difference was significant for both standard and diverse forages. Under the optimum irrigation regime, there was no significant difference in herbage DM yield or N uptake between urine application rates and forage types (Figure 4.8 and Figure 4.9).

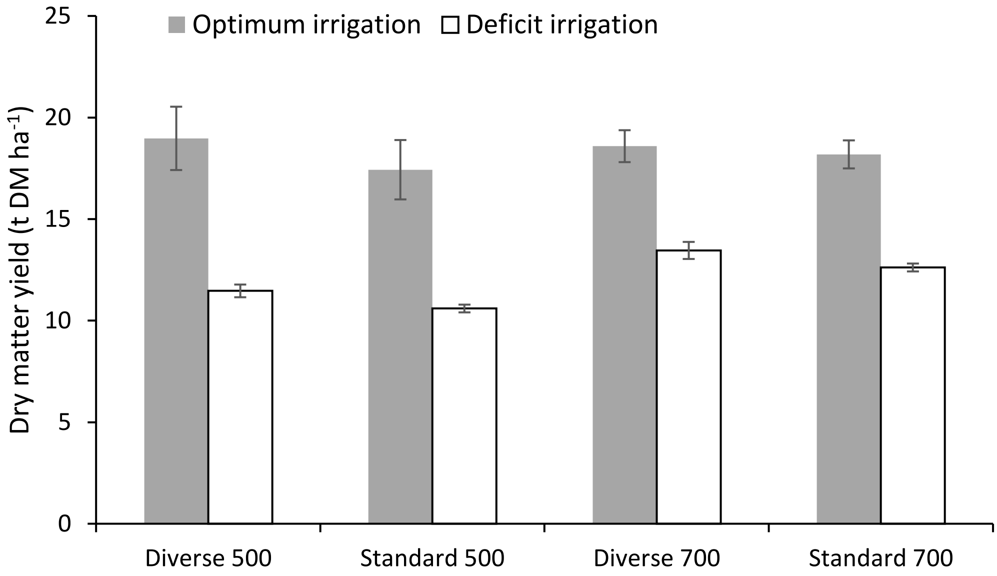


Figure 4.8. Total herbage DM yield (t DM ha^{-1}) as affected by irrigation (optimum vs. deficit), plant species (standard vs. diverse) and urine patch loading rate (500 vs. 700 kg N ha^{-1}). Error bars are ± SEM. Number of replicates = 5.

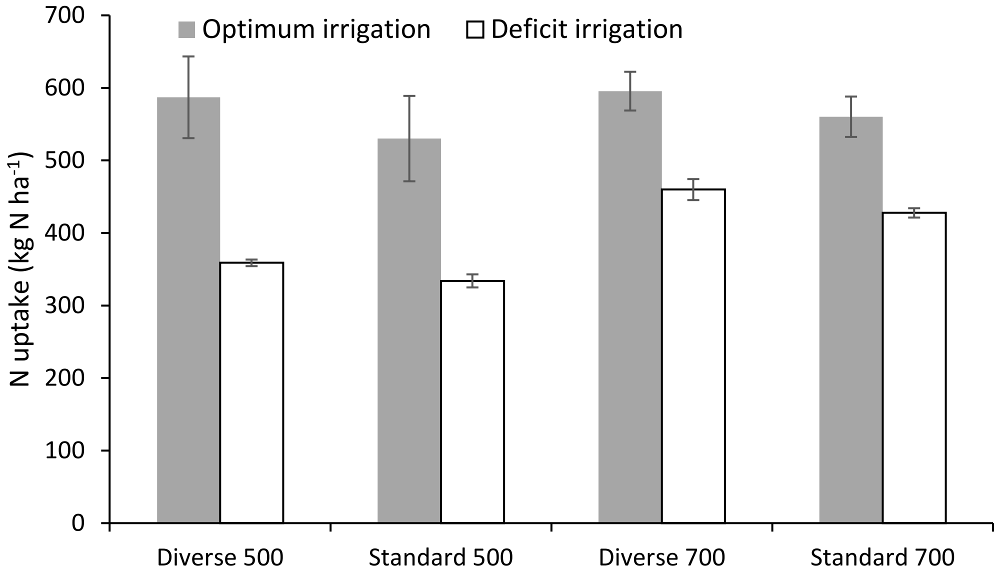


Figure 4.9. Total herbage N uptake (kg N ha^{-1}) as affected by irrigation (optimum vs. deficit), plant species (standard vs. diverse) and urine patch loading rate (500 vs. 700 kg N ha^{-1}). Error bars are ± SEM. Number of replicates = 5.

4.3.5 Botanical composition

The proportion of each plant species in the standard forage is illustrated in Figure 4.10. The majority of the standard forage (62–96%) was perennial ryegrass, with the remainder being clover (2–32%) and weed species (<2%). The proportion of each plant species in the diverse forage is illustrated in Figure 4.11. For all treatments, except the optimum irrigation 500 treatment, perennial ryegrass was the dominant species (25–56%). Under the optimum irrigation 500 treatment, clover was the dominant species (46%), which was greater than the other treatments (7–24%). The proportion of plantain (8–13%) was greater compared to the proportion of chicory (1–5%). The clover proportion was mainly white clover with little red clover present.

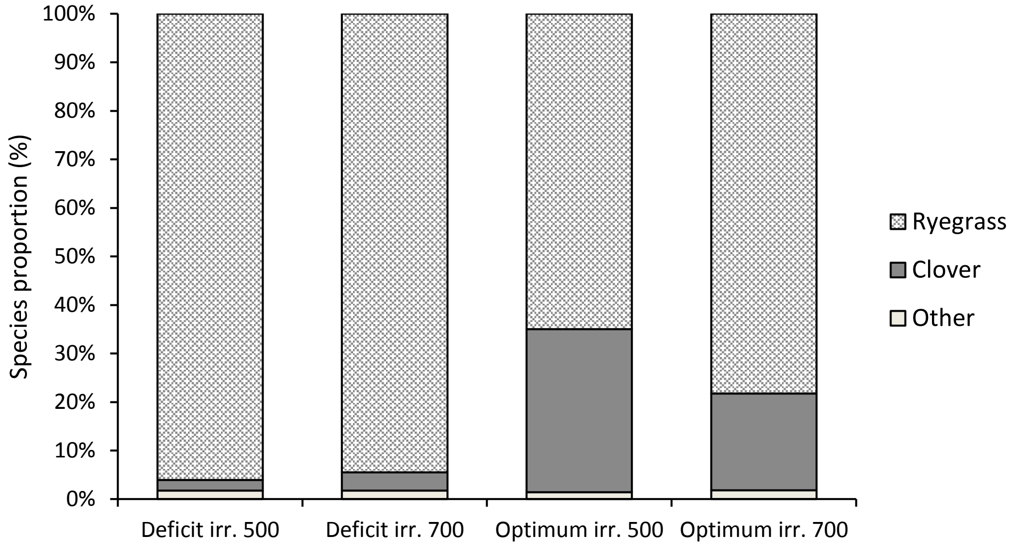


Figure 4.10. Proportion of species in the standard forage containing perennial ryegrass and white clover. Herbage was harvested 14 April 2015.

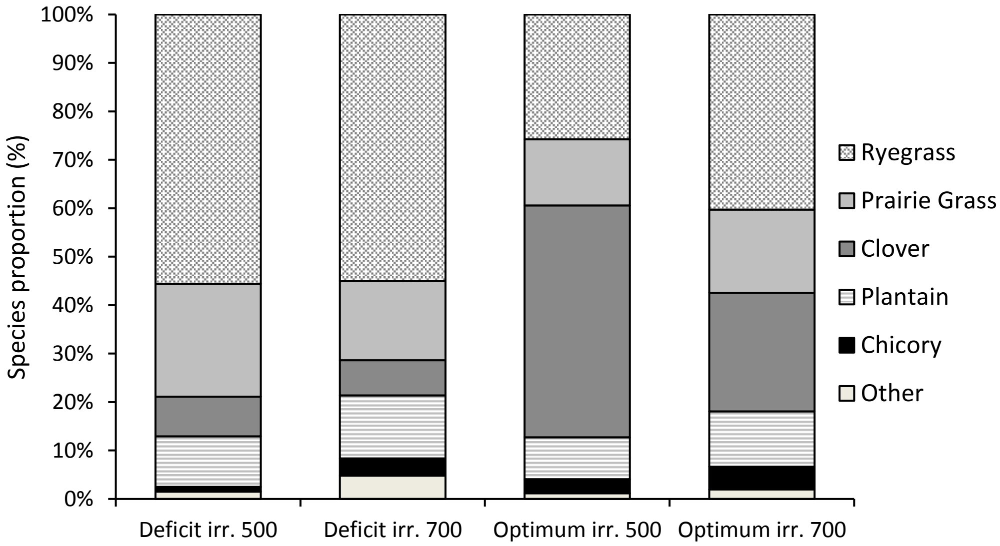


Figure 4.11. Proportion of species in the diverse forage containing perennial ryegrass, white clover, prairie grass, plantain, chicory. Herbage was harvested 14 April 2015.

4.3.6 Seasonal herbage yield and N uptake

Seasonal herbage DM yield ranged from 0.9 t DM ha^{-1} (winter) to 5.2 t DM ha^{-1} (autumn) under deficit irrigation, and 0 t DM ha^{-1} (winter) to 12.1 t DM ha^{-1} (summer) under optimum irrigation (Figure 4.12). In summer, the optimum irrigation produced 6–8 t DM ha^{-1} more dry matter than the deficit irrigation ($P > 0.05$). In winter, the deficit irrigation produced 0.9–2.1 t DM ha^{-1} more dry matter than the optimum irrigation ($P > 0.05$) (Figure 4.12). Averaged across all treatments, herbage DM yield was affected by forage type in all seasons with a significant interaction occurring between irrigation type and urine rate in autumn, winter and spring (Table 4.6).

Seasonal herbage N uptake ranged from 21.8 kg N ha^{-1} (winter) to 185.0 kg N ha^{-1} (autumn) under deficit irrigation, and 0 kg N ha^{-1} (winter) to 408.5 kg N ha^{-1} (summer) under optimum irrigation (Figure 4.13). Under optimum irrigation, in summer, herbage N uptake was 166–270 kg N ha^{-1} greater than deficit irrigation ($P > 0.05$). In contrast, in winter, deficit irrigation herbage N uptake was 22–64 kg N ha^{-1} greater than the optimum irrigation ($P > 0.05$) (Figure 4.13). Averaged across all treatments, herbage N uptake was affected by forage type in all seasons with a significant interaction occurring between irrigation type and urine rate in autumn, winter and spring, and forage type and urine rate in winter, spring and summer (Table 4.6).

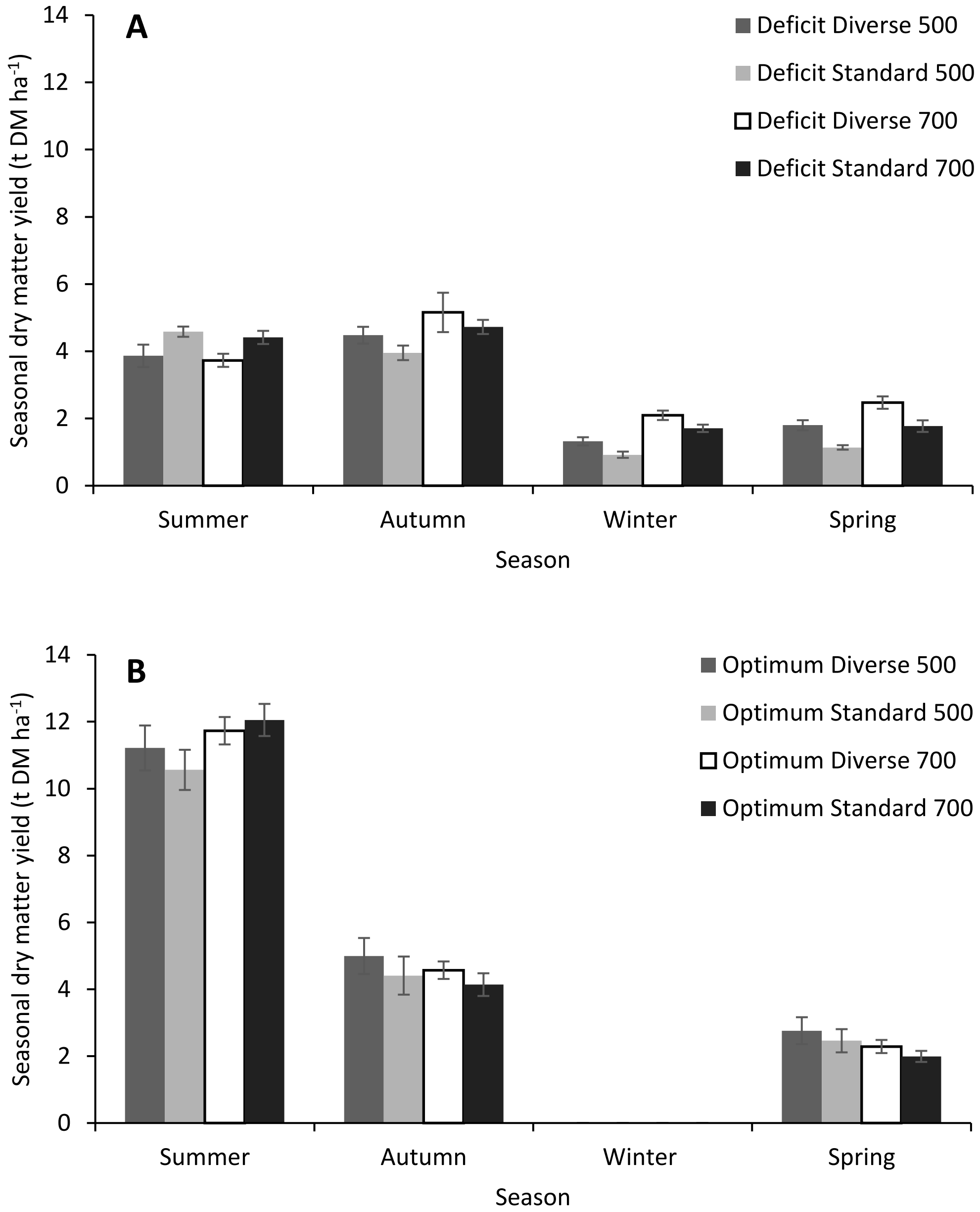
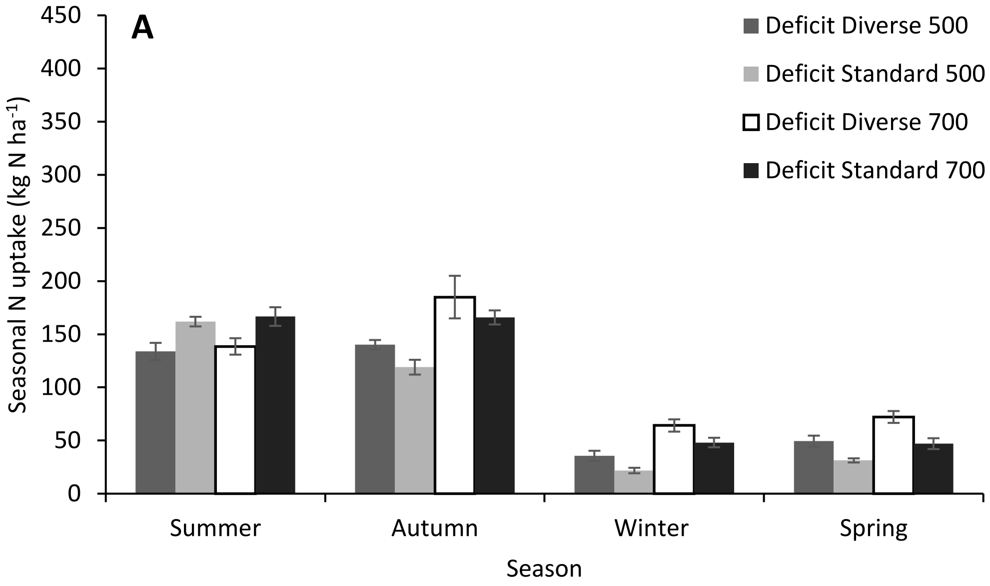


Figure 4.12. Seasonal herbage DM yield (t DM ha^{-1}) as affected by deficit irrigation (A), optimum irrigation (B), and plant species (standard vs. diverse) and urine patch loading rate (500 vs. 700 kg N ha^{-1}). Error bars are ± SEM. Number of replicates = 5.



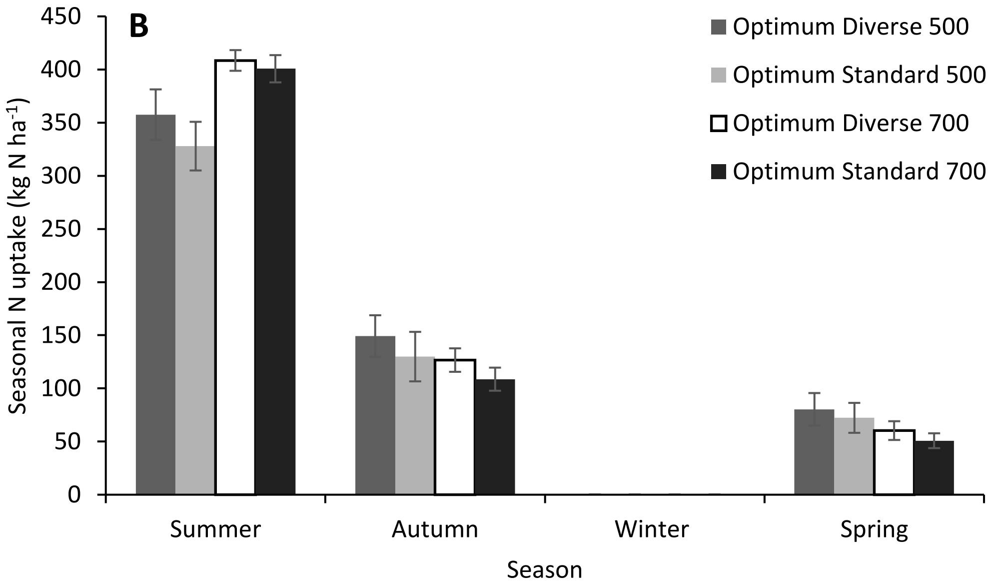


Figure 4.13. Seasonal herbage N uptake (kg N ha^{-1}) as affected by deficit irrigation (A), optimum irrigation (B), and plant species (standard vs. diverse) and urine patch loading rate (500 vs. 700 kg N ha^{-1}). Error bars are ± SEM. Number of replicates = 5.

Table 4.6. Seasonal herbage DM yield (kg DM ha^{-1}) and herbage N uptake (kg N ha^{-1}) from lysimeters during the experimental period.

			Log$_{10}$ means							
			DM yield (kg DM ha^{-1})				N uptake (kg N ha^{-1})			
Irrigation	Forage	Urine rate	Sum	Win	Aut	Spr	Sum	Win	Aut	Spr
Deficit	Diverse	500	3.580	3.114	3.648	3.250	2.123	1.536	2.146	1.684
	Standard	500	3.660	2.956	3.594	3.053	2.209	1.327	2.072	1.492
	Diverse	700	3.570	3.317	3.703	3.388	2.139	1.800	2.259	1.853
	Standard	700	3.643	3.229	3.673	3.240	2.220	1.673	2.218	1.662
Optimum	Diverse	500	4.047	0	3.689	3.424	2.549	0	2.161	1.875
	Standard	500	4.021	0	3.631	3.377	2.512	0	2.091	1.832
	Diverse	700	4.068	0	3.657	3.353	2.612	0	2.097	1.764
	Standard	700	4.080	0	3.611	3.293	2.602	0	2.027	1.688
LSD (5%) within irrigation regimes			0.061	0.065	0.072	0.088	0.056	0.089	0.076	0.103
LSD (5%) for all other comparisons			0.067	0.073	0.104	0.125	0.064	0.100	0.121	0.170
Significance of main effect										
Irrigation			***	***	NS	*	***	***	NS	NS
Forage			*	***	*	***	*	***	**	***
Urine rate			NS	***	NS	NS	**	***	NS	NS
Significance of interaction										
Irrigation × forage			**	***	NS	**	***	***	NS	*
Irrigation × urine rate			NS	***	*	***	*	***	***	***
Forage × urine rate			NS	NS	NS	NS	NS	NS	NS	NS
Irrigation × forage × urine rate			NS	NS	NS	NS	NS	NS	NS	NS

NS = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

4.3.7 ^{15}N recovery

Leachate

The total amount of ^{15}N recovered under the standard forage was 1.8% compared with 3.2% under the diverse forage (Figure 4.14), however there was no statistically significant effect on total leachate ^{15}N recovery (%) (P = 0.486).

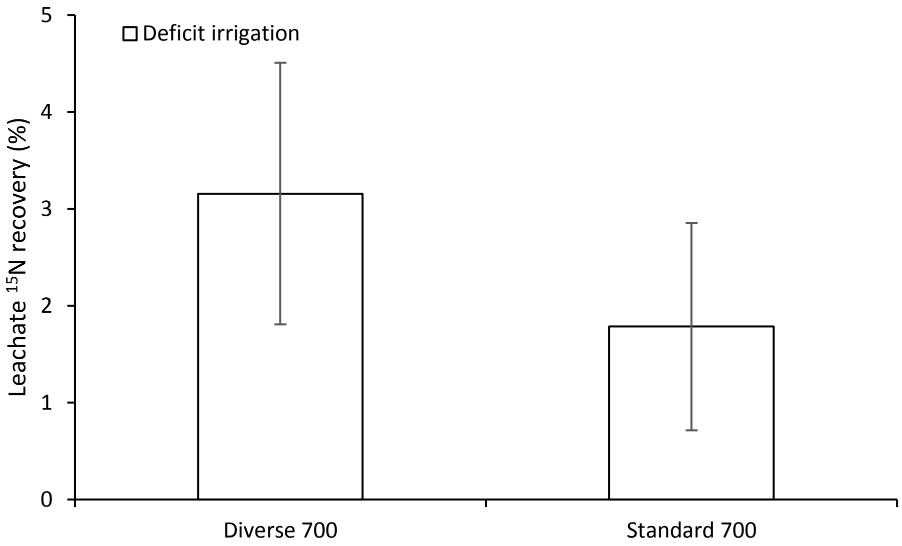


Figure 4.14. Leachate ^{15}N recovery as affected by forage type (standard vs. diverse) under deficit irrigation at a loading rate of 700 kg N ha^{-1}. Number of replicates = 3.

Herbage

The majority of ^{15}N recovered in the herbage occurred in the first 50 days following urine application (Figure 4.15). The highest herbage ^{15}N recovery occurred under the optimum irrigation treatment with 26% recovered by the standard forage under the 500 kg N ha^{-1} urine treatment on day 23 (Figure 4.15).

Averaged across all treatments, total herbage ^{15}N recovery (%) was strongly influenced by irrigation ($P < 0.001$) and forage type ($P = 0.019$). Total herbage ^{15}N recovery under optimum irrigation (47–50%) was higher than under deficit irrigation (37–40%). This represented a significant ($P < 0.05$) increase in herbage ^{15}N recovery of 22–28% under optimum irrigation (Figure 4.16). Under both irrigation regimes, there was no significant difference in herbage ^{15}N recovery between urine application rates ($P = 0.762$).

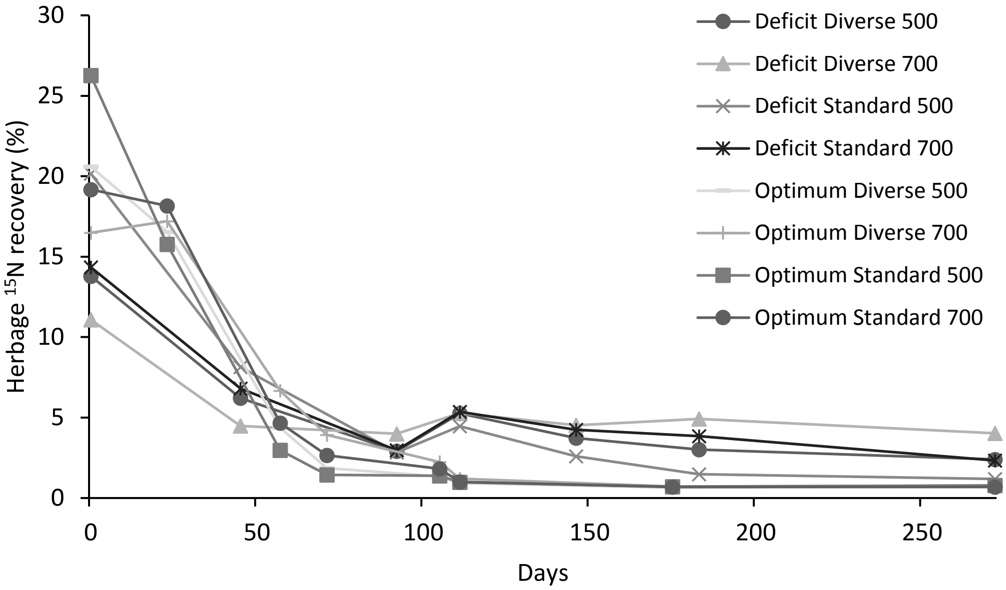


Figure 4.15. Herbage ^{15}N recovery as affected by irrigation (optimum vs. deficit), plant species (standard vs. diverse) and urine patch loading rate (500 vs. 700 kg N ha^{-1}).

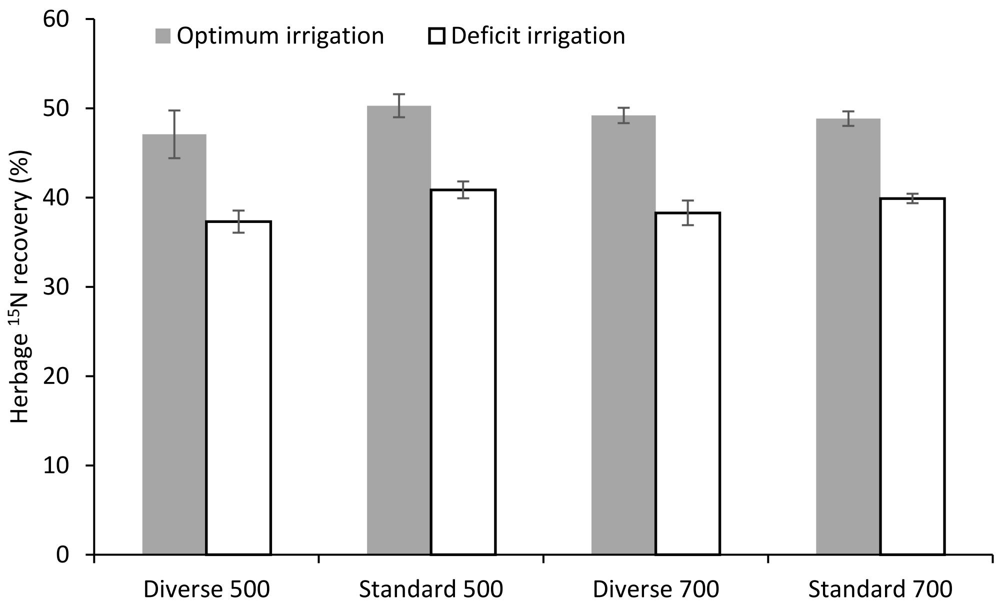


Figure 4.16. Total herbage ^{15}N recovery as affected by irrigation (optimum vs. deficit), plant species (standard vs. diverse) and urine patch loading rate (500 vs. 700 kg N ha^{-1}). Error bars are ± SEM. Number of replicates = 5.

4.4 Discussion

4.4.1 Nitrate leaching losses

The results from this experiment show that, over a 10 month period, NO_3^-–N leaching losses from spring deposited urine (applied at a rate of 700 kg N ha^{-1}) were 88–97% lower under optimum irrigation compared with deficit irrigation. The lower NO_3^-–N leaching losses from the optimum irrigation treatments were mainly a result of greater herbage N uptake and consequently lower soil mineral N concentrations remaining in the soil. This is in agreement with the findings of Snow and White (2013), who modelled the effect of herbage growth rate and water stress on NO_3^- leaching losses from urine (750 kg N ha^{-1}) deposited by grazing dairy cows using APSIM (Agricultural Production Systems Simulator). Nitrate leaching was reported to be consistently lower from irrigated forages than from dryland forages, regardless of higher fertiliser applications in the irrigation treatment, and was attributed to greater herbage growth rates over the summer period. Snow and White (2013) also found that under drought conditions, a low level of water stress (WF_5) compared with a medium (WF_3) and a high level of water stress (WF_1), greatly reduced the amount of soil mineral N by 67 and 118 kg N ha^{-1} six months after spring urine deposition, and resulted in lower NO_3^- leaching losses by 44 ($WF_1 – WF_3$) and 61 ($WF_3 – WF_5$) kg N ha^{-1}. Other studies have also reported greater NO_3^- leaching losses in winter following a summer drought (Webster & Dowdell, 1984; Scholefield *et al.*, 1993; Cuttle *et al.*, 1998; Stout *et al.*, 2000). This was attributed to greater accumulation of soil mineral N in dry years resulting from low herbage growth, the release of N from dying roots and nodules, and an increased flush of mineralisation from soil rewetting.

At both the 500 and 700 kg N ha^{-1} urine application rates, the total NO_3^-–N leaching losses from spring deposited urine were small when either optimum or deficit irrigation was applied over the summer period (Figure 4.7). These values were notably lower than NO_3^- leaching losses reported in previous findings, and were also substantially less than those reported from autumn deposited urine (Di & Cameron, 2002c; Decau *et al.*, 2003; Buckthought *et al.*, 2015). For example, Buckthought *et al.* (2015) reported that NO_3^-–N leaching losses from spring deposited urine (800 kg N ha^{-1}) were 91% lower than the losses from autumn applied urine. These results suggest that the contribution of NO_3^-–N leaching losses is small (even when water was limited over the summer period). Forage N uptake over the summer period was likely the main reason for the low NO_3^- leaching losses observed from the spring deposited urine. The warm temperatures, adequate soil moisture (under optimum irrigation) and longer daylight hours suggest that forage growth over this period would have been uninhibited (Brougham, 1959). Therefore, because the urine was applied when herbage was growing rapidly, there was a greater chance of N being taken up. This is supported by Di and Cameron (2002b) who found that N uptake from spring deposited urine was just as high or higher than N uptake from urine deposited earlier in the year in autumn. The low NO_3^-–N leaching losses observed under the deficit irrigation may be the result of a flush of herbage growth that occurred in late autumn/early winter due to warm temperatures and an increase in soil moisture. An increase in the recovery of urinary–N in herbage under deficit irrigation was observed during the late autumn period in this study, and suggests that under drought conditions, urine deposited in spring will remain in the active root zone over the summer period. Simpson (1962) also demonstrated that mineral N can accumulate in the soil during summer droughts when herbage growth is low, thus it is probable that this combined with favourable growing conditions resulted in the late season uptake of soil mineral N, particularly under the deficit irrigation regime. Under these conditions, it is also possible that N was lost through denitrification (Di & Cameron, 2003; Phillips *et al.*, 2007), which could have further reduced the amount of N available for leaching from spring deposited urine. However, while favourable autumn conditions promoted late N uptake in this experiment, it also highlights the risk of N leaching from urine deposited late spring following drought conditions or poor irrigation management over the summer period.

4.4.2 Herbage DM yield and N uptake

Herbage DM production under deficit irrigation was lower than under optimum irrigation for both forage types, and highlights the possibility of increasing forage production through good irrigation management in grazed systems. Neal *et al.* (2009) reported similar herbage yields, under deficit and optimum irrigation regimes, for a variety of species that received N fertiliser at a rate of 800 kg N ha^{-1} over the duration of the trial, however, unlike this experiment the forages were grown as monocultures. Neal *et al.* (2009) also found that although deficit irrigation reduced herbage yield, there was a large variation between forages in tolerance to water deficits. While 5 of the 12 forages in the study by Neal *et al.* (2009) had a significantly higher mean DM yield than perennial ryegrass under deficit irrigation, under optimum irrigation this was reduced to 3 of the 15 forage species. Woodward *et al.* (2013) found that the DM yield (averaged over three years), was similar in standard (15.3 t DM ha^{-1} yr^{-1}) and diverse (14.7 t DM ha^{-1} yr^{-1}) forages grown in the Waikato region. Malcolm *et al.* (2014) also reported no significant difference in the amount of NO_3^-–N leached between diverse forages (containing herbs plantain and chicory) and standard perennial ryegrass and white clover forages when urine was applied at a rate of 700 kg N ha^{-1}. Furthermore, studies have shown there to be no difference in the root architecture of herbs plantain and chicory, and perennial ryegrass (Skinner & Comas, 2010; Malcolm *et al.*, 2014), which could possibly account for the similar NO_3^- leaching losses that were observed under diverse and standard forages in this experiment. Alternatively, cool season growth and root density rather than the presence of tap roots may be of greater importance to enhance the capture of soil mineral N. This is supported by Habib and La Folie (1991) who suggested that forages with a greater density of roots in the upper soil depths could have a greater potential to capture soil N than those at depth, and Malcolm *et al.* (2014) who reported a reduction in NO_3^- leaching loss under winter active Italian ryegrass (*Lolium multiflorum* Lam.) and white clover forages when compared to a perennial ryegrass and white clover forage.

4.4.3 ^{15}N recovery

The aim of this experiment was not to generate a complete ^{15}N balance but to use the ^{15}N tracer to compare the recovery of urinary–N in herbage and in leachate under the different experimental treatments. However, it is worth considering pathways other than herbage and leachate N recovery in which the urinary–N was utilised or lost too. Urinary ^{15}N recovery in herbage was greater under optimum irrigation than under deficit irrigation, with the majority recovered directly after the urine application and over the summer period. Decau *et al.* (2003) and Buckthought *et al.* (2016), reported similar ^{15}N recovery from urine deposited in spring, with 58% and 52% of urine deposited N recovered in the herbage, respectively. With the recovery of urinary–N in the herbage being as much as 50% of applied N, this represents a major removal of deposited N from the urine patch under optimum irrigation. The large plant N uptake of urinary–N is therefore likely to be responsible for the low NO_3^- leaching (< 4 kg N ha^{-1}) that was observed under the optimum irrigation regime.

The recovery of ^{15}N in the leachate was low (< 4%) and reflected the overall low total NO_3^- leaching under the deficit irrigation with a loading rate of 700 kg N ha^{-1}. Low ^{15}N recovery in the leachate was also observed by Decau *et al.* (2003) who reported a leachate ^{15}N recovery of 0.7% from spring deposited urine. For autumn applied urine, ^{15}N recovery in leachate has been reported to range from 6.4–13.1% under a perennial ryegrass and white clover forage (Fraser *et al.*, 1994; Clough *et al.*, 1998; Di *et al.*, 2002). Following urine application in November, 8.4 mm of rain fell within 12 h of the urine application. So it is unlikely that a large portion of urinary–N was lost via volatilisation (Black *et al.*, 1987). While not measured in this experiment it is also likely that a portion of the urinary–N applied was immobilised in the soil. The recovery of ^{15}N in the soil has been reported to range from 16.5–31.0% (Fraser *et al.*, 1994; Clough *et al.*, 1998; Di *et al.*, 2002; Decau *et al.*, 2003; Leterme *et al.*, 2003). Plant roots may also account for up to 19.2% of ^{15}N recovery (Di *et al.*, 2002). Denitrification may also have resulted in the loss of urinary–N which was applied. However, Clough *et al.* (1998) reported ^{15}N recovery from nitrous oxide emissions to account for only 1% of the total ^{15}N recovered from cow urine.

4.4.4 Effect of urinary–N concentration

There was a trend towards NO_3^- leaching losses from diverse forages at a loading rate of 500 kg N ha^{-1} being lower than losses from the standard forage at a loading rate of 700 kg N ha^{-1} for both irrigation regimes. Di and Cameron (2007) have shown that NO_3^-–N leaching losses increased significantly from 59.7 to 188.1 and 254.9 kg NO_3^-–N ha^{-1} when urine N was applied at a rate of 300, 700 and 1000 kg N ha^{-1}. Similar increases in NO_3^-–N leaching losses have also been observed with increasing rates of N fertiliser (Barraclough *et al.*, 1992; Wachendorf *et al.*, 2004). Recent studies have shown the urinary–N concentration of cows grazing a diverse forage to be lower than those grazing a standard perennial ryegrass and white clover forage (Woodward *et al.*, 2012; Box *et al.*, 2016; Bryant *et al.*, 2017). It is therefore possible that a reduction in urinary–N excretion, from cows grazing a diverse forage, may result in lower NO_3^-–N leaching losses due to lower N inputs into the grazed forage system. Reducing the urinary–N loading rate by grazing cows on diverse forages could therefore be an effective mitigation option to help reduce NO_3^-–N leaching losses. The proportion of herbs in the diverse forage ranged from 8–13% (plantain) and 1–5% (chicory). A greater proportion of herbs in the forage may therefore be required to achieve a significant reduction in NO_3^-–N leaching losses.

4.5 Conclusions

The main conclusions drawn from this experiment are:

- Nitrate leaching losses were lower from the forages that received optimum irrigation compared with those that received the deficit irrigation regime. This was attributed to greater herbage DM yield and N uptake during the summer period by forages that were not water limited under optimum irrigation.

- These results demonstrate the potential for good irrigation management to reduce NO_3^- leaching losses from spring deposited urine in grazed forages. Good irrigation management is one strategy that could easily be integrated into farm practices to reduce the impact of agriculture on the environment.

Chapter 5

Effect of irrigation type and forage type on nitrification under cow urine patches

5.1 Introduction

5.1.1 Biological nitrification inhibition

Nitrate (NO_3^-) leaching from grazed forage systems is a well-known environmental concern. This subject has been reviewed in detail by Di and Cameron (2002a), Ledgard *et al.* (2011) and Cameron *et al.* (2013). Nitrification, a microbial process mediated by ammonia oxidising bacteria (AOB) and archaea (AOA), determines the amount of NO_3^- present in the soil and therefore how N is utilized or dispersed into the environment (Di *et al.*, 2009; Di *et al.*, 2010). The nitrification of urine, deposited by grazing cattle in particular, is responsible for high concentrations of NO_3^- in soil solution (Selbie *et al.*, 2015). The use of nitrification inhibitors is one possible approach to mitigating NO_3^- leaching losses (Di & Cameron, 2016). Nitrification inhibition with commercially produced nitrification inhibitors (i.e. dicyandiamide) applied to soil treated with urine has been well documented (Di & Cameron, 2002b; Di & Cameron, 2007; Di *et al.*, 2010). Recently, however, there has been increased interest in the use of forage species capable of producing plant secondary metabolites (PSM) which suppress nitrifying microbes in the plant root rhizosphere and the surrounding soil. This is known as biological nitrification inhibition (BNI) (Subbarao *et al.*, 2006).

Biological nitrification inhibition has been observed under a number of plant species including the tropical forage grass *Brachiaria humidicola*, perennial grass *Hyparrhenia diplandra* and *Brassica* species (Lata *et al.*, 2000; Subbarao *et al.*, 2007a; Brown & Morra, 2009; Subbarao *et al.*, 2009). Recently, studies by Rauber *et al.* (2008), Dietz *et al.* (2013) and Massaccesi *et al.* (2015) have also reported nitrification inhibition when the forage species plantain (*Plantago lanceolata*) is present. Dietz *et al.* (2013) found that the application of aucubin (a PSM found in plantain) to soil resulted in lower NO_3^-–N accumulation and higher NH_4^+–N accumulation compared with control treatments. Similarly, in field plots sown with plantain and two other grassland species (*Anthoxanthum odoratum* and *Lotus corniculatus*), Massaccesi *et al.* (2015) observed a reduction in soil NO_3^-–N concentration under plots where plantain was the dominant species, and attributed this to both mineralisation and nitrification being inhibited. This potential BNI effect of plantain is of particular interest because plantain is being recommended for incorporation into diverse forage mixtures in New Zealand. This is a potential way to reduce the nitrogen (N) concentration in animal urine in order to reduce the risk of NO_3^- leaching losses from the animal urine patches associated with standard perennial ryegrass (*Lolium* *perenne* L.) and white clover (*Trifolium repens*) forages (Pembleton *et al.*, 2015; Box *et al.*, 2016; Bryant *et al.*, 2017).

Hypothesis: Nitrification rates will be lower under diverse forages containing plantain compared with standard perennial ryegrass and white clover forages due to the release of biological nitrification inhibiting compounds by plantain.

5.1.2 Irrigation type

Currently, there is little known about how diverse forages containing plantain respond to the different irrigation types or how different irrigation types may affect nitrification rates in soil. Three main types of irrigation are used in New Zealand: (i) pivot/spray irrigation; (ii) rotorainer irrigation; and, to a lesser extent, (iii) flood irrigation (Irrigation New Zealand, 2017). While it has been shown that soil moisture content significantly affects the growth of nitrifiers in urine treated soils (Sänger *et al.*, 2011; Di *et al.*, 2014), it is not well understood how different irrigation types may affect the growth of these key microbial populations.

5.1.3 Objectives

The objectives of this experiment were therefore to: (i) determine AOB and AOA abundance under standard perennial ryegrass and white clover forages, and diverse forages containing plantain following cow urine deposition, and the subsequent effect on nitrification and (ii) determine the effect of three different irrigation types (pivot, rotorainer and flood) on nitrifier abundance and the subsequent effect on nitrification.

This chapter describes the soil block experiment which was conducted in parallel with the lysimeter experiment (Chapter Six). The results from this experiment will be used to help interpret the leaching results from Chapter Six.

5.2 Materials and methods

5.2.1 Experiment description, forage type and preparation

Twenty four soil blocks were installed alongside 30 lysimeters in the Lincoln University Research Dairy Farm (LURDF) field facility (Plate 5.1). The lysimeters and soil blocks were taken from irrigated plots sown in a standard or a diverse forage on LURDF (Figure A.1). The plots were mown periodically (c. monthly) to a residual height of 50 mm (c. 1500 kg DM ha^{-1}) prior to the lysimeters and soil blocks being collected. From the time the plots were sown the area was not grazed.

Plate 5.1. Fully installed soil blocks in the field facility.

The standard and diverse forages were sown in March 2014 and at the time of extraction were two years old. The standard forage contained perennial ryegrass and white clover. The diverse forage contained perennial ryegrass, white clover and plantain. Sowing rates and cultivars are shown in Table 5.1. The perennial ryegrass cultivar used was 'Trojan' with NEA2 endophyte. Trojan is a late heading and high yielding cultivar with high production on the shoulders of the season (winter/early spring and autumn) (Agriseeds Ltd, 2013). Visual observations in the field and photographs of individual lysimeters and soil blocks were used to estimate the proportion of plantain in the diverse forage. It was estimated that the proportion of plantain in the diverse forage ranged from 20–30% (Plate 5.2).

Table 5.1. Species, cultivars and sowing rates for standard and diverse forages.

Plant species	Scientific name	Cultivar	Sowing rate (kg seeds ha^{-1})	
			Standard forage	Diverse forage
Perennial ryegrass	*Lolium perenne* L.	Trojan	20	20
White clover	*Trifolium repens* L.	Kopu ll	3	3
Plantain	*Plantago lanceolata* L.	Tonic	0	3

Plate 5.2. Diverse forage lysimeter (top left) and soil block (top right) containing perennial ryegrass, white clover and plantain. Diverse forage site prior to lysimeter collection (bottom left) and a standard forage lysimeter containing perennial ryegrass and white clover (bottom right).

Prior to the soil blocks being taken, the remaining forage at the collection plots received the same treatments as the fully installed lysimeters. Maintenance and urea fertiliser applications replicated those applied to the lysimeters. Herbage from the collection plots was mown to a height of 50 mm (c. 1500 kg DM ha^{-1}) and removed. The field plots were irrigated regularly with a boom sprinkler irrigator to ensure plant growth was not moisture limited over the summer period.

To determine the fate of urine applied in late summer (February), dairy cow urine was applied to the lysimeters and the soil blocks on the 19 February 2016. The soil blocks enabled destructive sampling to be carried out for microbial analysis following the February urine application, while simultaneously, measurements of NO_3^- leaching losses were made from the undisturbed lysimeters.

5.2.2 Soil block collection

Intact soil blocks (500 mm diameter × 75 mm deep), were collected from the standard and diverse forage plots. A cylindrical metal casing was carefully pushed into the soil in small 20 mm increments. Once at the desired depth, a cutting plate was then used to separate the soil block from the underlying subsoil. The soil blocks were then installed alongside the lysimeter facility at LURDF. A metal sheet (150 mm deep) was then inserted at the half way point of the soil block, and sat flush with the metal casing (Plate 5.3). This prevented urine seeping into the control portion of the soil block. The soil blocks were installed on top of a sandy layer to promote natural drainage. The trench was then back filled with soil to the same level as the soil block surface. This enabled the soil blocks to be exposed to the same climatic conditions as the surrounding field.

Plate 5.3. Fully installed soil block with dividing metal barrier down the centre.

5.2.3 Experimental design

The experimental design consisted of twelve treatments including three irrigation types (pivot vs. rotorainer vs. flood), two forage types (standard vs. diverse), one urinary–N application rate (700 kg N ha^{-1}) and a control (no urine) (Table 5.2). Treatments were arranged in a randomised design and were replicated four times. Dairy cow urine was applied on the 19 February 2016 and measurements were taken thereafter until the 16 May 2016.

Table 5.2. Description of soil block treatments.

Treatment no.	Irrigation type	Irrigation rate (mm)	Irrigation frequency interval (days)	Forage type	Urine application date	Urine treatment (kg N ha^{-1})
1	Pivot	15	3	Standard	February	700
2	Pivot	15	3	Diverse	February	700
3	Rotorainer	45	9	Standard	February	700
4	Rotorainer	45	9	Diverse	February	700
5	Flood	90	18	Standard	February	700
6	Flood	90	18	Diverse	February	700
7	Pivot	15	3	Standard	February	0
8	Pivot	15	3	Diverse	February	0
9	Rotorainer	45	9	Standard	February	0
10	Rotorainer	45	9	Diverse	February	0
11	Flood	90	18	Standard	February	0
12	Flood	90	18	Diverse	February	0

5.2.4 Urine application

On the 18 February 2016 (late summer), fresh dairy cow urine was collected from Friesian × Jersey cross cows on the Lincoln University Dairy Farm (LUDF) during the afternoon milking. The total urinary–N concentration was 5.2 g N L^{-1}. This was standardised to a concentration of 7 g N L^{-1} using urea, and glycine in a 9:1 ratio (Bathurst, 1952). One half of the soil block received a 1 L surface application of urine to simulate typical urine patch deposition by grazing dairy cows at a loading rate of 700 kg N ha^{-1} (Selbie *et al.*, 2015) (Plate 5.4). The remaining half of the soil block received a 1 L water application to maintain a similar soil moisture content across all treatments.

Plate 5.4. Cow urine application to one half of the soil block.

5.2.5 Irrigation scheduling

From February to April (summer) pivot and rotorainer irrigation treatments were simulated using the irrigation system described in Section 3.7. Flood irrigation was manually applied to individual soil blocks and lysimeters during this period. For the soil blocks this involved measuring out two 9 L volumes of water (equivalent to 90 mm) and pouring each of the 9 L volumes onto one half of the soil block to simulate flood irrigation. A stainless steel ring was attached to the soil block to allow the large volume of water to be applied, and to prevent water overflowing from the lysimeter. The pivot irrigation treatment comprised of irrigation every three days at an application rate of 15 mm and at an intensity of 20 mm per hour (Table 5.2). The rotorainer irrigation treatment comprised of irrigation every nine days at an application rate of 45 mm and at an intensity of 20 mm per hour, and the flood irrigation treatment comprised of irrigation every 18 days at an application rate of 90 mm and at an intensity of 90 mm per hour (Table 5.2). Irrigation was applied to match typical irrigation rates for pivot, rotorainer and flood irrigation in the Canterbury region (Hydroservices Ltd, 2014). The soil moisture sensors described in Section 3.6.3 were used to monitor the soil moisture content and adjust irrigation applications when necessary. In the event of natural rainfall, the period of time between irrigation events was extended to remain consistent with typical irrigation practice.

5.2.6 Fertiliser

Prior to treatment application, all soil blocks and lysimeters received a maintenance fertiliser application in the form of 20% potash super sulphur (6.4–10–16–14) equivalent to 65 kg P ha^{-1} (standard forage) and 50 kg P ha^{-1} (diverse forage). The rate of fertiliser applied was determined from soil test results (Table 3.2). Urea fertiliser was applied in split applications to provide an annual rate of 125 kg N ha^{-1}. As per recommended farm practice, N fertiliser was applied between October and April in split applications of 25 kg N ha^{-1} (Fertiliser Assosiation, 2009). All fertiliser was hand applied evenly across the surface of the soil block. This was followed with 10 mm of irrigation to wash the fertiliser into the soil and to prevent volatilisation.

5.2.7 Soil block sampling

Three soil cores (75 mm depth) were randomly taken from both the urine and control treatments within each soil block (Plate 5.5). Samples were collected on day 1, 7, 14, 30, 60 and 90 for DNA extractions, potassium chloride (KCl) extractions and soil moisture content. The three soil cores were bulked, thoroughly mixed, and sub-samples were taken. All control blocks were sampled first to avoid contamination and the corer was cleaned between each block. Extra soil taken from the soil block collection site was used to backfill the remaining holes. Markers were then used to avoid re-sampling from the same area (Plate 5.5). Herbage was cut on the same day as the lysimeters and discarded.

Plate 5.5. Soil cores were taken from the control and urine side of the soil block (left), and the holes were back filled with soil and marked (right).

5.2.8 Analysis

5.2.8.1 Soil analysis

Ammonium and nitrate

Potassium chloride extracts were carried out on 5 g of field moist soil using 25 mL of 2M KCl extraction solution (Keeney & Nelson, 1982). The soil samples were thoroughly mixed prior to analysis, and a sub-sample was oven-dried at 105°C to enable correction for soil moisture content. The KCl extract and soil suspension were shaken for 1 hour on an end over shaker and then centrifuged at 4000 rpm for 10 minutes. Once removed, the sample was filtered through a 110 mm Avantec 5C filter paper funnel, and frozen at -20°C until analysed (See Section 4.2.6.1). Soil inorganic N concentrations (NO_3^- and NH_4^+) were determined using a FOSS FIAstar 5000 twin channel analyser (Foss Tecator AB, Hoganas, Sweden).

Soil moisture content

A sub-sample was taken on each sampling day to measure the moisture content of each bulked soil sample. Approximately 10 g of soil was taken, oven-dried at 105°C for 24 hours, and reweighed. The soil moisture was determined using Equation 7:

$$\text{Soil moisture (\%)} = ((\text{wet soil (g)} - \text{dry soil (g)}) \times 100)/\text{dry soil (g)} \tag{7}$$

5.2.8.2 AOA and AOB assays

Soil samples were collected on day 1, 7, 14, 30, 60 and 90 and stored at -80°C until DNA extraction. The DNA extraction and PCR analysis followed methodologies adapted from Di *et al.* (2009).

DNA extraction

DNA was extracted from the soil using a NucleoSpin® Soil Kit (Macherey-Nagel, Düren, Germany) as per the manufacturer's instructions. In brief, a 0.25 g soil sample was weighed into a NucleoSpin® Bead Tube to which 700 µL of Buffer SL2, and 150 µL of Enhancer SX was added. The sample was homogenised for 1 minute using a FastPrep®-24 Sample Preparation System (M.P. Biomedicals, California, USA) at a speed of 6 m s^{-1}. The tubes were centrifuged at 11000 rpm for 2 minutes (Centrifuge 5424, Eppendorf AG, Hamburg, Germany), and the supernatant transferred into a sterilised 1.7 mL tube. Next 150 µL of Buffer SL3 was added and the samples were shaken for 5 seconds prior to incubation at 4°C for 5 minutes. The tubes were centrifuged at 11000 rpm for 1 minute, and up to 700 µL of supernatant was transferred into a NucleoSpin® Inhibitor Removal Column fitted on top of a collection tube. Tubes were again centrifuged at 11000 rpm for 1 minute and the column was discarded. 250 µL of Buffer SB was added to the flow through and mixed with a pipette. A NucleoSpin® Soil Column was placed in a new collection tube and a 550 µL sample of this was loaded onto the column. This was centrifuged at 11000 rpm for 1 minute and the flow through discarded. This step was repeated until no sample remained. 500 µL of Buffer SB was added to the NucleoSpin® Soil Column. This was centrifuged at 11000 rpm for 30 seconds and the flow through was again discarded. The column was then washed with 550 µL of Buffer SW1, and twice with 700 µL of Buffer SW2. Once the final flow through was discarded, the column and collection tube was centrifuged at 11000 rpm for 2 minutes to remove any residual ethanol. The NucleoSpin® Soil Column was then transferred to a new collection tube and the DNA was eluted using 100 µL of Elution Buffer SE. The sample was incubated at room temperature for 1 minute and centrifuged at 11000 rpm for 30 seconds. DNA was stored at -20°C for further analysis.

PCR analysis

AOA and AOB ammonia monoxygenase gene (*amo*A) abundance was measured using real time quantitative polymerase chain reaction (qPCR) on a Rotor-Gene™ 6000 (Corbett Research, Australia). All qPCR reactions were prepared using a CAS1200 Robotic liquid handling system (Corbett Robotics, Australia). The AOA and AOB *amo*A genes were quantified using the primer pairs amoA-1F and amoA R-I (Hornek *et al.*, 2006), and Arch-amoAF and Arch-amoAR (Francis *et al.*, 2005), respectively (Table 5.3). A reaction mixture of 16 µL contained 8 µL 2x SYBR® Premix Ex Taq™ (Tli RNaseH Plus, Takara Bio Inc., Shiga, Japan), 0.4 µL of each primer, and sterile deionised water to bring up to total volume of 14.5 µL and 1.5 µL of DNA sample. All genomic DNA samples were all diluted ten times with deionised water prior to use. Serial dilutions of standards with a range of 10^1 to 10^7 copies μL^{-1} were run in duplicate for each gene to produce standard curves. Once the PCR reactions were prepared the RotorDisc™ 100 was sealed using a Gene-Disc™ Heat Sealer (HS-01, Corbett Research, Australia). The qPCR temperature profiles used are given in Table 5.3. A melting curve analysis was performed after amplification to check for nonspecific amplification products. The fluorescence was measured continuously as the temperature increased from 72°C to 99°C. Data were then analysed using the Rotor-Gene™ series software 1.7.

Standard curves for real-time qPCR were developed using the following process. Bacterial and archaeal *amoA* genes were amplified from the extracted DNA using the aforementioned primers. A qPCR clean up kit (Axygen) was used to purify the PCR products which were cloned into the pGEM-T Easy Vector (Promega, Madison, WI). Following the manufacturer's instructions, the resulting clones were transformed in *Escherichia coli* JM109 competent cells (Promega). The transformed *E. coli* cells were grown on solid LB plates at 37°C overnight. Ten to fifteen bacterial colonies from the plate were individually inoculated into a 3 mL LB broth medium and incubated overnight in an orbital incubator-shaker at 37°C and 250 rpm. The plasmids carrying correct gene inserts were then extracted from bacterial cultures using QIA Prep Spin Miniprep Kit (Qiagen, Crawley, UK) and sent for sequencing. The DNA concentration was determined on a Qubit™ Fluorometer (Invitrogen™, New Zealand). The copy numbers of target genes were calculated directly from the concentration of extracted DNA. To generate an external standard curve, tenfold serial dilutions of a known copy number of the extracted plasmid DNA were subjected to a real-time PCR assay in duplicate.

Table 5.3. qPCR primers and temperature cycles (Francis *et al.*, 2005; Hornek *et al.*, 2006)

		AOA *amoA*		**AOB *amoA***	
Primer pairs		Arch-amoAF		amoA-1F	
		5' STAATGGTCTGGCTTAGACG-3'		5'-GGGGHTTYTACTGGTGGT-3'	
		Arch-amoAR		amoA R-i	
		5'-GCGGCCATCCATCTGTATGT-3'		5'-CCCCTCNGNAAANCCTTCTTC-3'	
# of cycles	**Cycling conditions**	**Temp. (°C)**	**Time (s)**	**Temp. (°C)**	**Time (s)**
1	Initial denaturation	94	120	94	120
	Denaturation	94	20	94	20
40	Primer annealing	55	30	57	30
	Extension	72	30	72	30

5.2.9 Statistical analysis

Statistical analysis was carried out using analysis of variance (ANOVA) using (Genstat 16th Edition, VSN International Ltd). Standard errors of the mean were calculated and presented with the mean values. The soil NO_3^- and ammonium (NH_4^+) concentration data and the AOA and AOB abundance data were log-transformed to normalise the variance and to determine statistical treatment effects.

5.3 Results

5.3.1 Temperature

Daily air and ground temperatures are given in Figure 5.1. The minimum air and ground temperatures were recorded on 29 April 2016 at 8.7°C and 11.5°C, respectively. The maximum air and ground temperatures were measured on 26 February 2016 and 17th February 2016 at 25°C and 22°C, respectively.

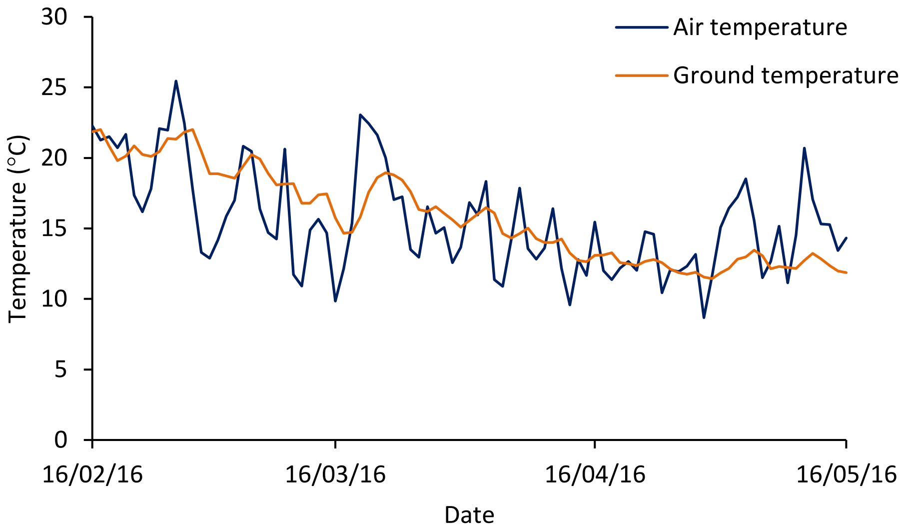


Figure 5.1. Average daily air and ground temperatures from February 2016 to May 2016.

5.3.2 Water inputs

From February 2016 to April 2016, the total amounts of irrigation applied were 254 mm under the pivot irrigation treatment (Figure 5.2A), 279 mm under the rotorainer irrigation treatment (Figure 5.2B), and 285 mm under the flood irrigation treatment (Figure 5.2C). The total rainfall (natural and simulated) for the experimental period was 106 mm, the majority of this fell in May 2016 (Figure 5.2).

As described in Section 5.2.5, each irrigation type was scheduled according to the amount of rainfall received (as would occur on farm with best practice irrigation management). To achieve this a model which accumulated daily climatic values as reference point was used to schedule irrigation events as described in Section 3.7.

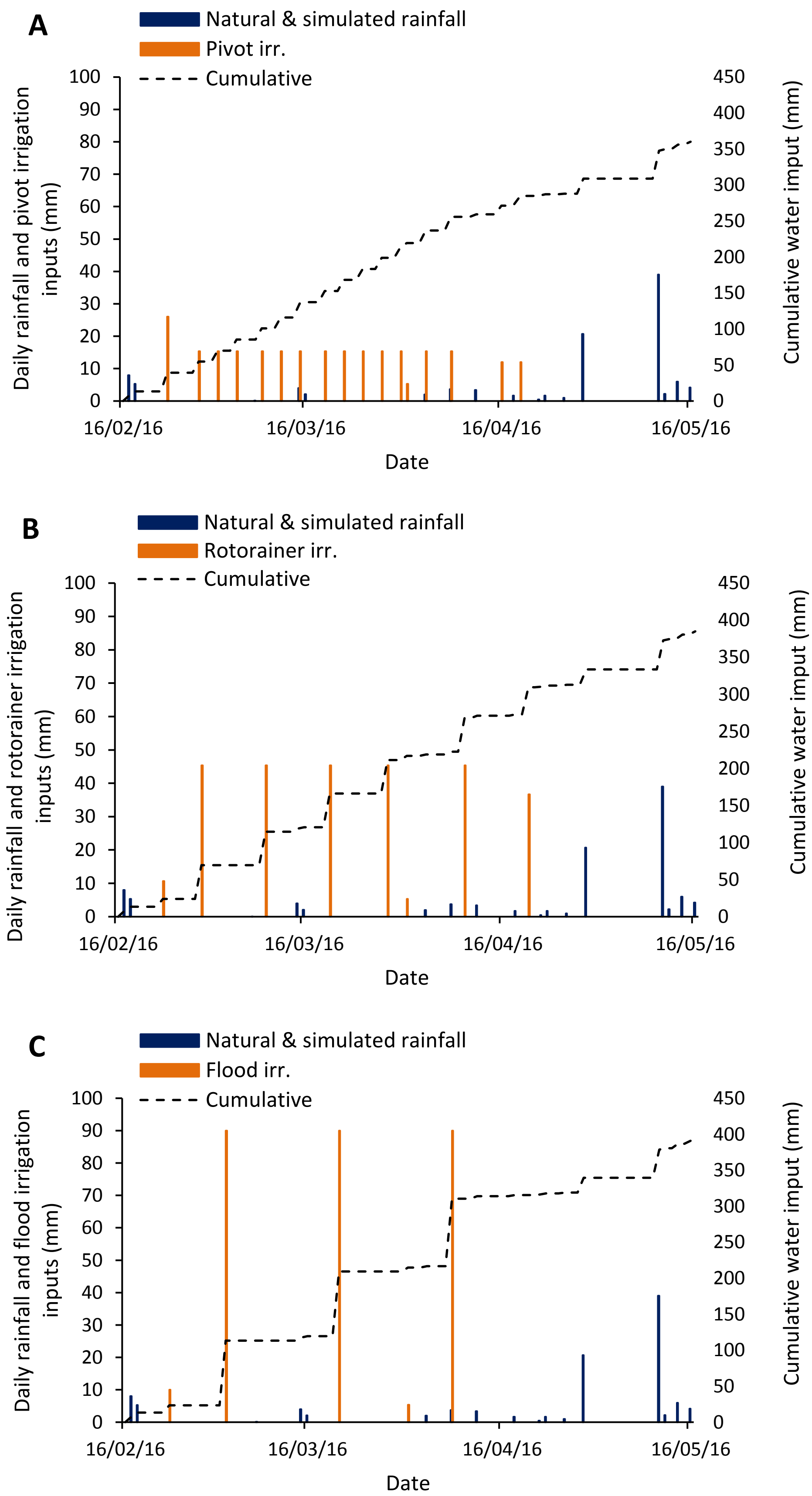


Figure 5.2. Cumulative and daily water inputs (mm) from February 2016 to May 2016 for pivot irrigation (A), rotorainer irrigation (B) and flood irrigation (C).

5.3.3 Effect of irrigation, forage and urine type

5.3.3.1 Soil ammonium and nitrate concentation

Under the different irrigation treatments, the peak soil NH_4^+–N concentration occurred on day 1 for soil blocks treated with urine and gradually decreased over time. There was no change in the soil NH_4^+–N concentration for the control treatments over the 90 day period (Figure 5.3). The soil NH_4^+–N concentration was significantly affected by irrigation type on days 1, 7, and 14 (Table 5.4). The diverse forage (day 1) had a significantly greater soil NH_4^+–N concentration under pivot irrigation compared with flood irrigation ($P < 0.05$) (Figure 5.3A & C). The standard forage (days 7 & 14) had a significantly greater soil NH_4^+–N concentration under flood irrigation compared with pivot irrigation ($P < 0.05$) (Figure 5.3A & C). The soil NH_4^+–N concentration was significantly affected by forage type on days 7, 14 and 30 (Table 5.4). The pivot irrigation (days 7 & 14), rotorainer irrigation (day 7 & 30) and flood irrigation (day 14) had significantly greater soil NH_4^+–N concentrations under the diverse forage compared with the standard forage ($P < 0.05$) (Figure 5.3A, B & C). The soil NH_4^+–N concentration was significantly affected by urine application on all days (Table 5.4 & Figure 5.3). There was little difference between treatments after day 30.

Under the different irrigation treatments, all urine treatments initially had low soil NO_3^-–N concentrations. The soil NO_3^-–N concentration increased over time and peaked between days 14 and 30 before gradually decreasing (Figure 5.4). There was no change in the soil NO_3^-–N concentration for the control treatment over the 90 day period (Figure 5.4). The soil NO_3^-–N concentration was significantly affected by irrigation on days 60 & 90 (Table 5.4). The standard and diverse forage types (day 60), had significantly greater soil NO_3^-–N concentrations under flood irrigation compared with pivot and rotorainer irrigation ($P < 0.05$) (Figure 5.4). The soil NO_3^-–N concentration was significantly affected by forage type on days 7, 14 and 90 (Table 5.4). The flood irrigation (day 7) had significantly greater soil NO_3^-–N concentrations under the standard forage compared with the diverse forage ($P < 0.05$) (Figure 5.4C). The soil NO_3^-–N concentration was significantly affected by urine application on days 7– 90 (Table 5.4 & Figure 5.4). There was little difference between treatments by day 90 for all irrigation types.

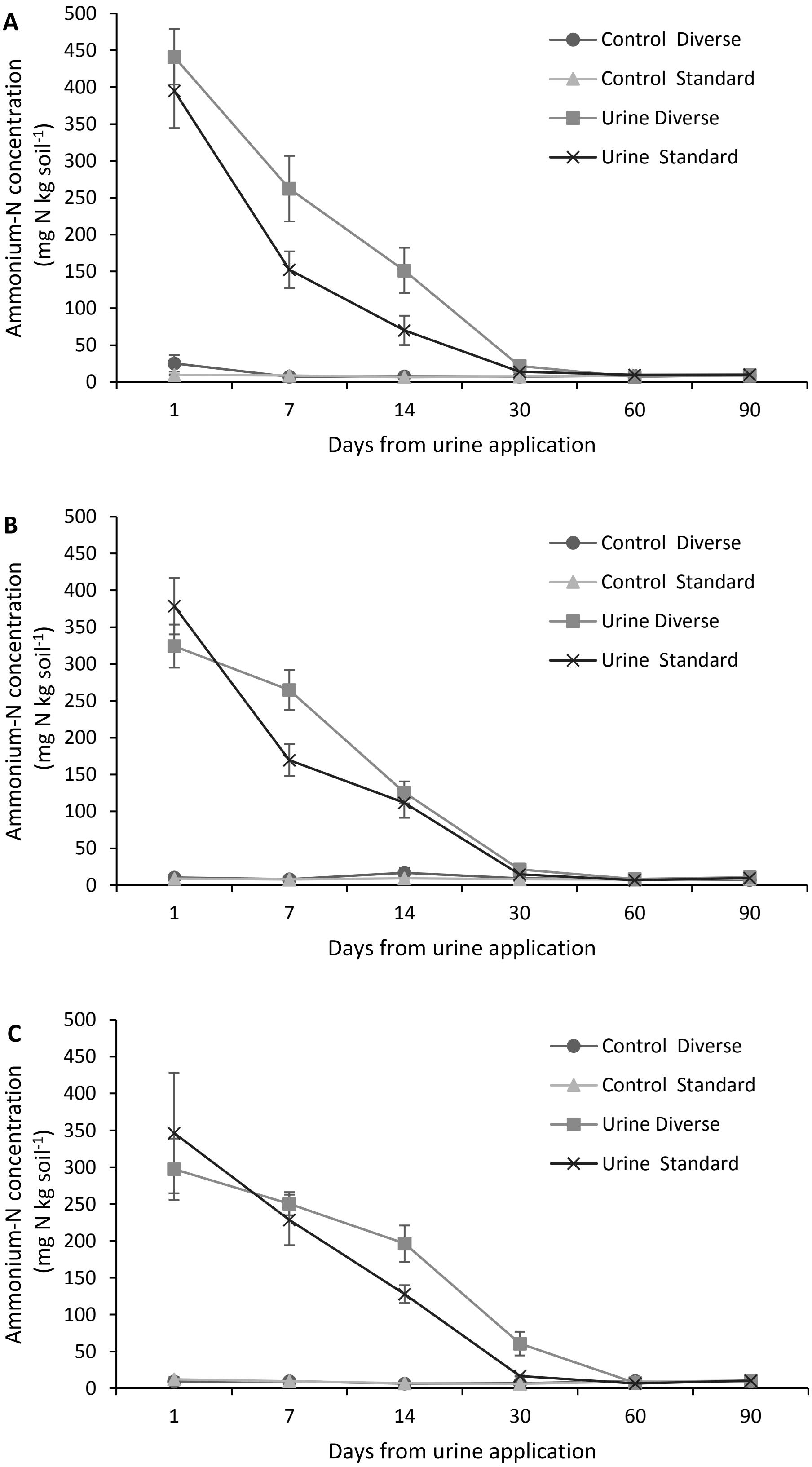


Figure 5.3. Ammonium–N concentrations (mg N kg soil^{-1}) in the soil as affected by pivot irrigation (A), rotorainer irrigation (B) and flood irrigation (C). Error bars are ± SEM.

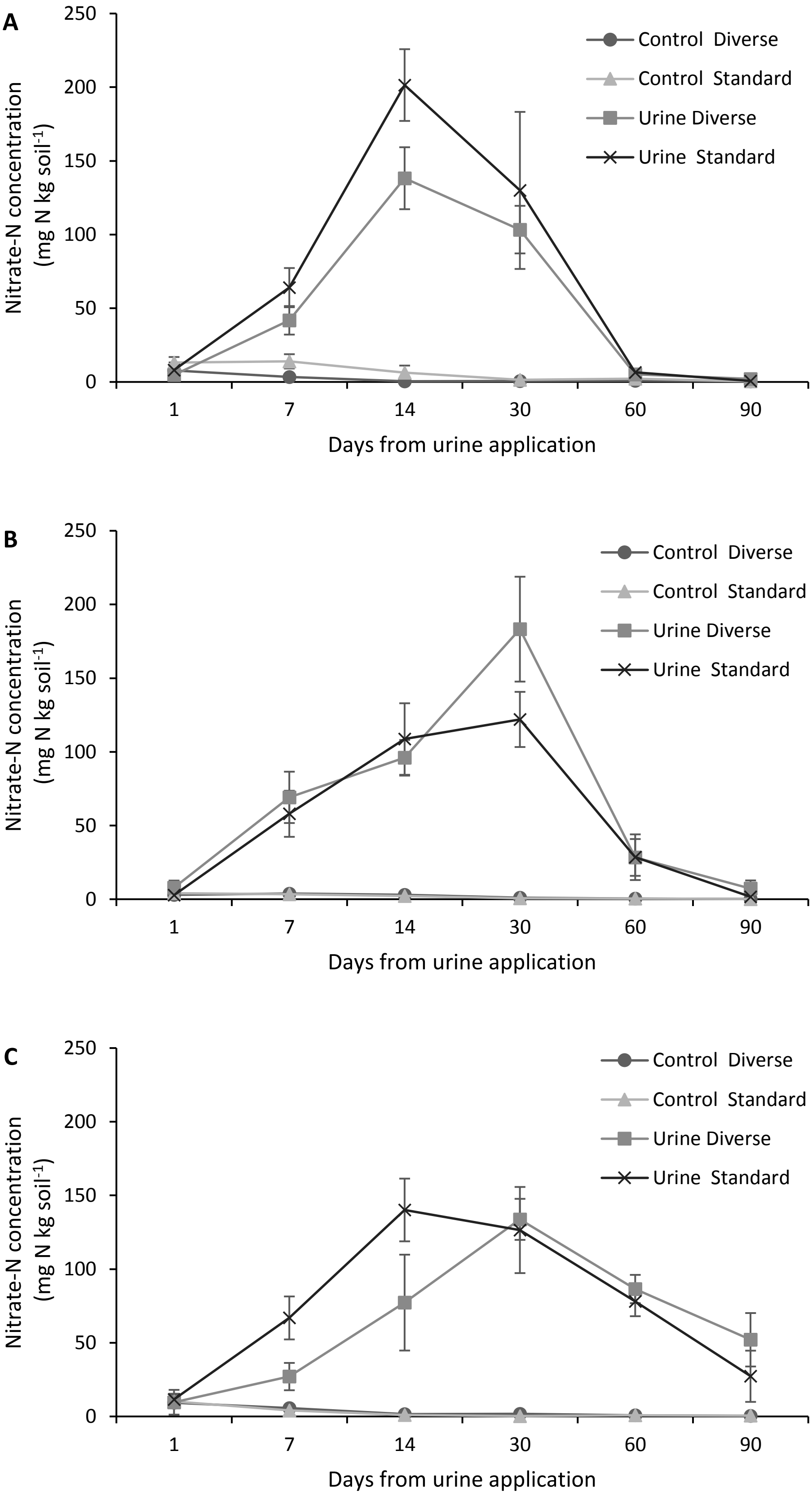


Figure 5.4. Nitrate–N concentrations (mg N kg soil^{-1}) in the soil as affected by pivot irrigation (A), rotorainer irrigation (B) and flood irrigation (C). Error bars are ± SEM.

Table 5.4. Soil ammonium–N concentrations and soil nitrate–N concentrations (mg N kg soil^{-1}) from day 1 to day 90 for urine + control soil blocks.

Irrigation	Forage	Treatment	Log$_{10}$ means: Soil ammonium concentration mg N kg soil^{-1}						Soil nitrate concentration mg N kg soil^{-1}					
			1	**7**	**14**	**30**	**60**	**90**	**1**	**7**	**14**	**30**	**60**	**90**
Pivot	Diverse	Control	1.298	0.847	0.878	0.872	0.930	0.947	0.615	0.492	-0.370	-0.249	-0.032	-0.016
	Standard	Control	0.986	0.949	0.812	0.877	0.943	0.947	1.069	1.080	0.421	0.118	0.158	-0.316
	Diverse	Urine	2.640	2.396	2.138	1.255	0.826	0.976	0.529	1.586	2.125	2.001	0.710	0.213
	Standard	Urine	2.585	2.165	1.798	1.078	0.982	1.006	0.852	1.772	2.293	2.025	0.679	-0.232
Rotorainer	Diverse	Control	1.020	0.902	1.155	0.960	0.900	0.863	0.459	0.515	0.238	-0.187	-0.528	-0.281
	Standard	Control	0.946	0.890	0.966	0.891	0.895	0.939	0.468	0.399	0.293	-0.298	-0.452	-0.684
	Diverse	Urine	2.506	2.417	2.091	1.211	0.921	1.030	0.692	1.803	1.972	2.238	1.299	0.483
	Standard	Urine	2.571	2.218	2.029	1.131	0.831	0.987	0.338	1.709	2.001	2.069	1.120	-0.171
Flood	Diverse	Control	0.967	0.985	0.811	0.848	0.980	0.975	0.558	0.648	0.171	0.043	-0.311	-0.552
	Standard	Control	1.055	0.978	0.827	0.783	0.964	1.015	0.913	0.597	-0.010	-0.578	0.071	-0.031
	Diverse	Urine	2.461	2.396	2.283	1.752	0.862	1.029	0.446	1.358	1.796	2.118	1.927	1.618
	Standard	Urine	2.515	2.348	2.102	1.222	0.819	1.020	1.000	1.803	2.135	2.081	1.886	0.978
LSD (5%) for all comparisons			0.204	0.142	0.210	0.300	0.129	0.102	0.576	0.379	0.472	0.377	0.556	0.736
<u>Significance of main effect</u>														
Irrigation			*	*	*	NS	NS	NS	NS	NS	NS	NS	***	***
Forage			NS	*	**	*	NS	NS	NS	*	*	NS	NS	*
Urine rate			***	***	***	***	*	**	NS	***	***	***	***	***
<u>Significance of interaction</u>														
Irrigation × forage			*	NS	NS	NS	NS	NS	NS	*	NS	*	NS	NS
Irrigation × urine rate			NS	NS	**	*	NS	NS	NS	NS	NS	NS	***	***
Forage × urine rate			NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Irrigation × forage × urine rate			NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS

NS = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

5.3.3.2 Soil AOB and AOA abundance

Under the different irrigation treatments, all urine treatments initially had low AOB *amoA* gene abundance. The AOB *amoA* gene abundance increased over time and peaked between days 30 and 60 before gradually decreasing. By comparison there was little change in the AOB *amoA* gene abundance for the control treatment over the 90 day period (Figure 5.5). There was a highly significant difference between AOB *amoA* gene abundance in the urine and control (non-urine) treatments after day 1 (Table 5.5). The soil AOB *amoA* gene abundance was also significantly affected by irrigation on days 14 & 30 (Table 5.5). The diverse forage (day 30) had significantly greater AOB *amoA* gene abundance under pivot irrigation compared with flood irrigation ($P < 0.05$) (Figure 5.5A & C). The soil AOB *amoA* gene abundance was significantly affected by forage type on days 1, 7, 14, 30 & 90 (Table 5.5). The flood irrigation treatment (days 7 & 30) had significantly greater AOB *amoA* gene abundance under the standard forage compared with the diverse forage ($P < 0.05$) (Figure 5.5C). The soil AOB *amoA* gene abundance was significantly affected by urine application on days 7–90 (Table 5.5 & Figure 5.5). There was little difference between treatments on days 1 & 90.

The AOB *amoA* gene abundance was greater than those of the AOA under all three irrigation types (Figure 5.5 & Figure 5.6). The soil AOA *amoA* gene abundance was significantly affected by irrigation on days 7 & 90 (Table 5.5). The standard forage (day 7) had significantly greater AOA *amoA* gene abundance under pivot irrigation compared with rotorainer irrigation ($P < 0.05$) (Figure 5.6A & B). The soil AOA *amoA* gene abundance was significantly affected by forage type on days 1–90 (Table 5.5). The pivot irrigation (day 7), rotorainer irrigation (days 14 & 30) and flood irrigation (days 7 &14) had significantly greater AOA *amoA* gene abundance under the diverse forage compared with the standard forage for the control treatments ($P < 0.05$) (Figure 5.6). All irrigation treatments (day 30) had significantly greater AOA *amoA* gene abundance under the diverse forage compared with the standard forage for the urine treatments ($P < 0.05$) (Figure 5.6).The soil AOA *amoA* gene abundance was significantly affected by urine application on days 7, 14, 30 and 90 (Table 5.5). Under urine application AOA *amoA* gene abundance was observed to be suppressed (Figure 5.6).

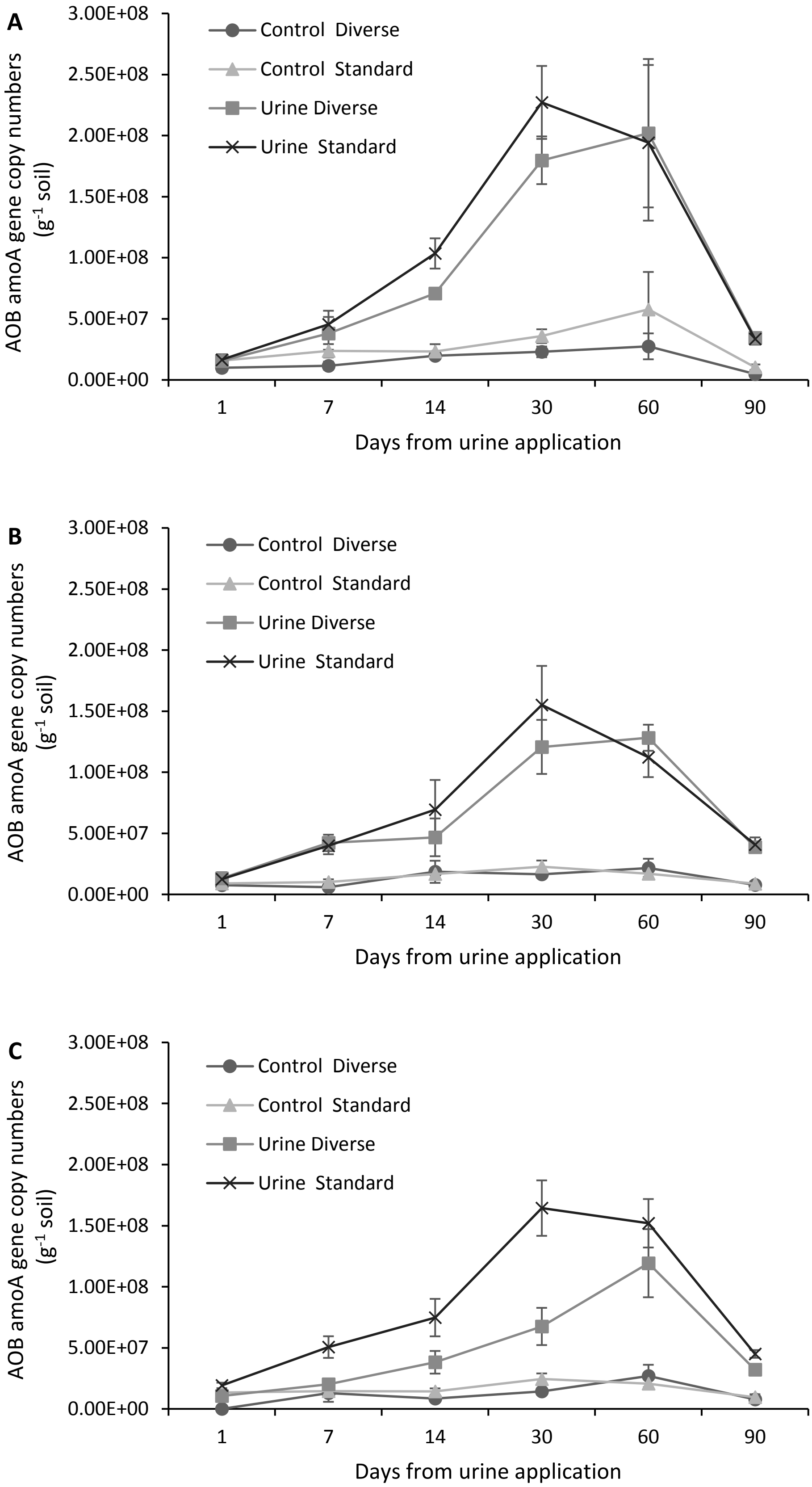


Figure 5.5. AOB *amoA* gene abundance (copy numbers g^{-1} soil) in the soil as affected by pivot irrigation (A), rotorainer irrigation (B) and flood irrigation (C). Error bars are ± SEM.

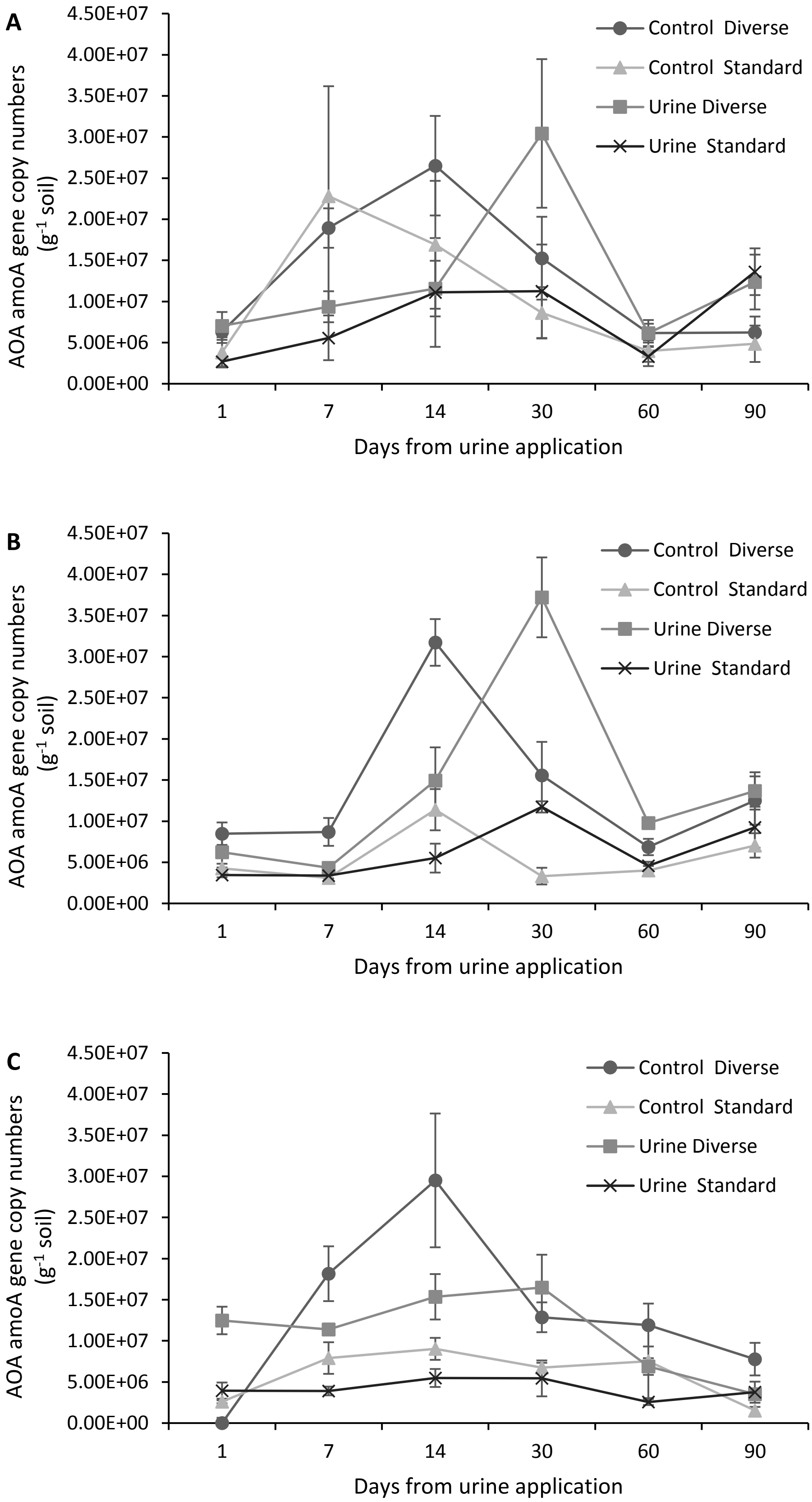


Figure 5.6. AOA *amoA* gene abundance (copy numbers g^{-1} soil) in the soil as affected by pivot irrigation (A), rotorainer irrigation (B) and flood irrigation (C). Error bars are ± SEM.

Table 5.5. Soil AOA and AOB *amoA* gene abundance (copy numbers g^{-1} soil) from day 1 to day 90 for urine + control soil blocks.

Irrigation	Forage	Treatment	Log$_{10}$ means AOA amoA gene copy numbers g^{-1} soil						AOB amoA gene copy numbers g^{-1} soil					
			1	7	14	30	60	90	1	7	14	30	60	90
Pivot	Diverse	Control	6.767	7.266	7.373	7.036	6.724	6.720	6.939	6.999	7.293	7.334	7.349	6.641
	Standard	Control	6.408	7.099	7.085	6.826	6.525	6.543	7.183	7.347	7.309	7.537	7.616	6.985
	Diverse	Urine	6.792	6.935	6.988	7.185	6.768	7.042	7.101	7.507	7.847	8.248	8.213	7.527
	Standard	Urine	6.400	6.577	6.799	6.599	6.452	7.100	7.210	7.611	8.005	8.344	8.182	7.511
Rotorainer	Diverse	Control	6.907	6.913	7.496	7.036	6.824	7.059	6.866	6.762	7.130	7.194	7.230	6.791
	Standard	Control	6.617	6.459	7.009	6.836	6.593	6.818	6.931	6.942	7.193	7.326	7.216	6.933
	Diverse	Urine	6.734	6.634	7.121	7.391	6.986	7.117	7.030	7.604	7.602	8.062	8.103	7.584
	Standard	Urine	6.533	6.522	6.566	6.097	6.652	6.919	7.057	7.582	7.689	8.164	8.036	7.595
Flood	Diverse	Control	6.803	7.233	7.426	7.097	7.047	6.838	7.131	6.941	6.883	7.128	7.341	6.845
	Standard	Control	6.411	6.865	6.946	6.826	4.714	6.148	7.127	7.156	7.138	7.374	7.295	6.952
	Diverse	Urine	7.085	7.054	7.168	7.185	6.824	6.524	6.912	7.273	7.540	7.791	8.038	7.502
	Standard	Urine	6.568	6.583	6.720	6.599	6.399	6.503	7.277	7.689	7.856	8.207	8.175	7.651
LSD (5%) for all comparisons			0.312	0.369	0.466	0.433	0.306	0.363	0.314	0.343	0.365	0.231	0.402	0.252
<u>Significance of main effect</u>														
Irrigation			NS	***	NS	NS	NS	***	NS	NS	*	***	NS	NS
Forage			***	***	***	***	***	**	*	**	*	***	NS	*
Urine rate			NS	**	***	*	NS	*	NS	***	***	***	***	***
<u>Significance of interaction</u>														
Irrigation × forage			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Irrigation × urine rate			NS	NS	NS	*	*	NS	NS	NS	NS	NS	NS	NS
Forage × urine rate			NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
Irrigation × forage × urine rate			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

5.3.4 Effect of forage type

To avoid bias between urine and control treatments, the urine treatment was examined on its own. This could be done because there was little to no effect between irrigation treatments. The data was therefore combined to examine the effect of forage type under the urine treatment. The urine treatment was considered more important in this experiment because it is a 'hotspot' for NO_3^- leaching loss.

5.3.4.1 Soil ammonium and nitrate concentration

The peak soil NH_4^+–N concentration occurred on day 1 for both the diverse (354 mg N kg soil^{-1}) and the standard forage (376 mg N kg soil^{-1}), and gradually decreased over time (Figure 5.7). Averaged across all urine treatments, the soil NH_4^+–N concentration was significantly affected by forage type on days 7 ($P = 0.006$), 14 ($P = 0.011$) and 30 ($P = 0.042$) (Table 5.6). The soil NH_4^+–N concentration under the standard forage was 30% (day 7), 39% (day 14) and 57% (day 30) lower than under the diverse forage ($P < 0.05$) (Figure 5.7).

The peak soil NO_3^-–N concentration occurred on day 14 under the standard forage (151 mg N kg soil^{-1}) but not until day 30 under the diverse forage (140 mg N kg soil^{-1}) (Figure 5.8). Averaged across all urine treatments, the soil NO_3^-–N concentration was significantly affected by forage type on days 14 ($P = 0.028$) and 90 ($P = 0.030$) (Table 5.6). The soil NO_3^-–N concentration under the diverse forage was 32% (day 14) lower than the standard forage ($P < 0.05$) (Figure 5.8). On day 90 the soil NO_3^-–N concentration was lower under the standard forage compared to the diverse forage ($P < 0.05$).

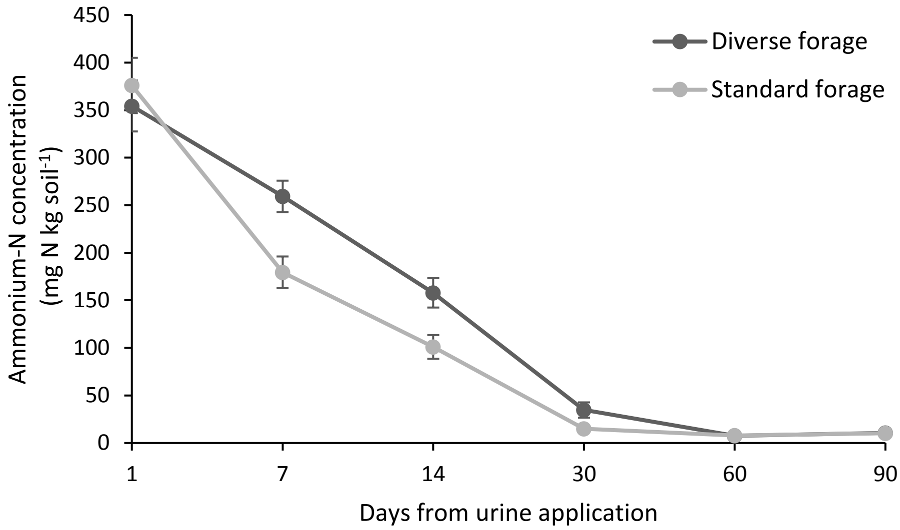


Figure 5.7. Mean ammonium–N concentrations (mg N kg soil^{-1}) in the soil as affected by forage type for the urine only treatments. Error bars are ± standard error of the mean (SEM).

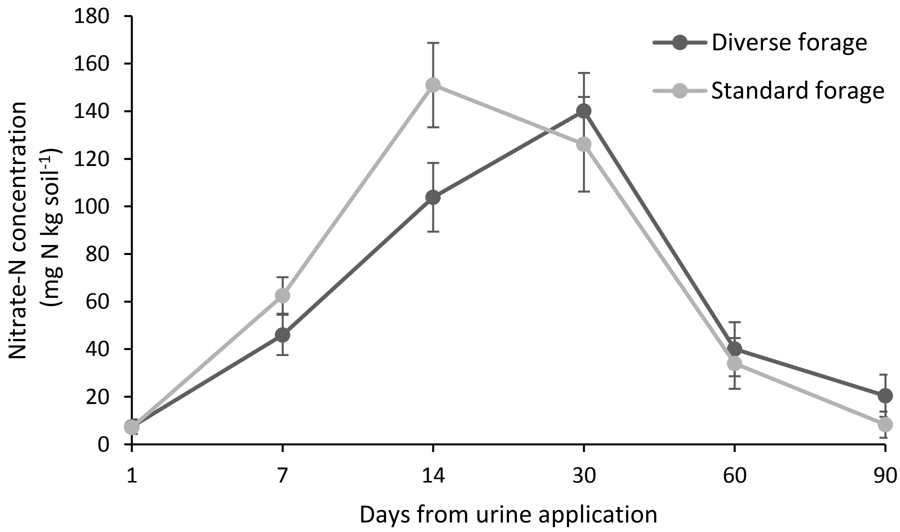


Figure 5.8. Mean nitrate–N concentrations (mg N kg soil^{-1}) in the soil as affected by forage type for the urine only treatments. Error bars are ± SEM.

Table 5.6. Soil ammonium–N and nitrate–N concentrations (mg N kg soil^{-1}) from day 1 to day 90 for urine only treated soil blocks.

			Log_{10} means											
Irrigation	**Forage**	**Treatment**	**Soil ammonium concentration mg N kg soil^{-1}**						**Soil nitrate concentration mg N kg soil^{-1}**					
			1	**7**	**14**	**30**	**60**	**90**	**1**	**7**	**14**	**30**	**60**	**90**
Pivot	Diverse	Urine	2.640	2.396	2.138	1.255	0.826	0.976	0.529	1.586	2.125	2.001	0.710	0.213
	Standard	Urine	2.585	2.165	1.798	1.078	0.982	1.006	0.852	1.772	2.293	2.025	0.679	-0.232
Rotorainer	Diverse	Urine	2.506	2.417	2.091	1.211	0.921	1.030	0.692	1.803	1.972	2.238	1.299	0.483
	Standard	Urine	2.571	2.218	2.029	1.131	0.831	0.987	0.338	1.709	2.001	2.069	1.120	-0.171
Flood	Diverse	Urine	2.461	2.396	2.283	1.752	0.862	1.029	0.446	1.358	1.796	2.118	1.927	1.618
	Standard	Urine	2.515	2.348	2.102	1.222	0.819	1.020	1.000	1.803	2.135	2.081	1.886	0.978
LSD (5%) for all comparisons			0.165	0.183	0.251	0.421	0.120	0.119	0.689	0.342	0.273	0.270	0.591	0.897
Significance of main effect														
Irrigation			NS	NS	*	NS	NS	NS	NS	NS	*	NS	***	***
Forage			NS	**	*	*	NS	NS	NS	NS	*	NS	NS	*
Significance of interaction														
Irrigation × forage			NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS

NS = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

5.3.4.2 Soil AOB and AOA abundance

The peak in AOB *amoA* gene abundance for all urine treatments occurred on day 30 under the standard forage (1.8×10^8 copies g^{-1} soil) and day 60 under the diverse forage (1.5×10^8 copies g^{-1} soil) (Figure 5.9). Averaged across all urine treatments, the AOB *amoA* gene abundance was significantly affected by forage type on day 30 ($P = 0.005$) (Table 5.7). The AOB *amoA* gene abundance under the diverse forage was 33% (day 30) lower than under the standard forage ($P < 0.05$) (Figure 5.9).

The peak in AOA *amoA* gene abundance for all urine treatments occurred on day 30 under both the standard forage (1.0×10^7 copies g^{-1} soil) and the diverse forage (2.8×10^7 copies g^{-1} soil) (Figure 5.10). The AOA *amoA* gene abundance under the diverse forage was greater than under the standard forage on days 1, 7, 14, 30 and 60 ($P < 0.05$) (Table 5.7).

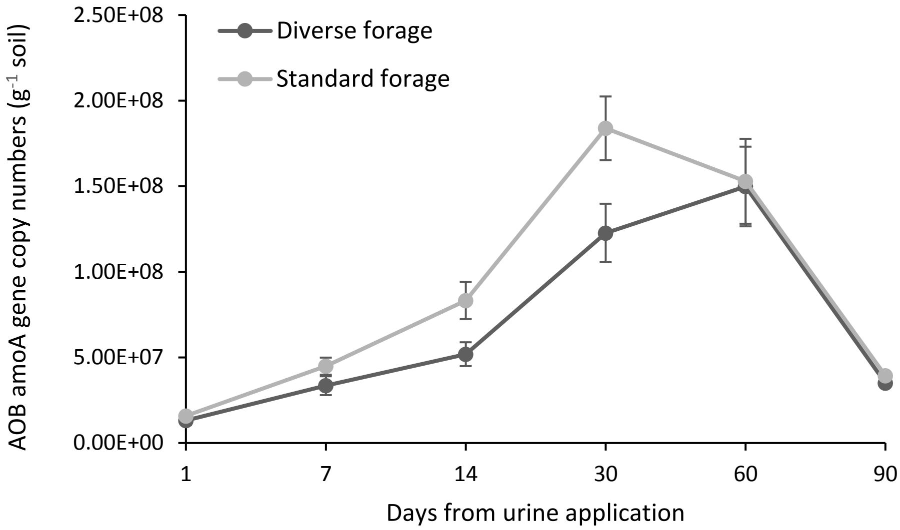


Figure 5.9. Mean AOB *amoA* (copy numbers g^{-1} soil) abundance in the soil as affected by forage type for the urine only treatments. Error bars are ± SEM.

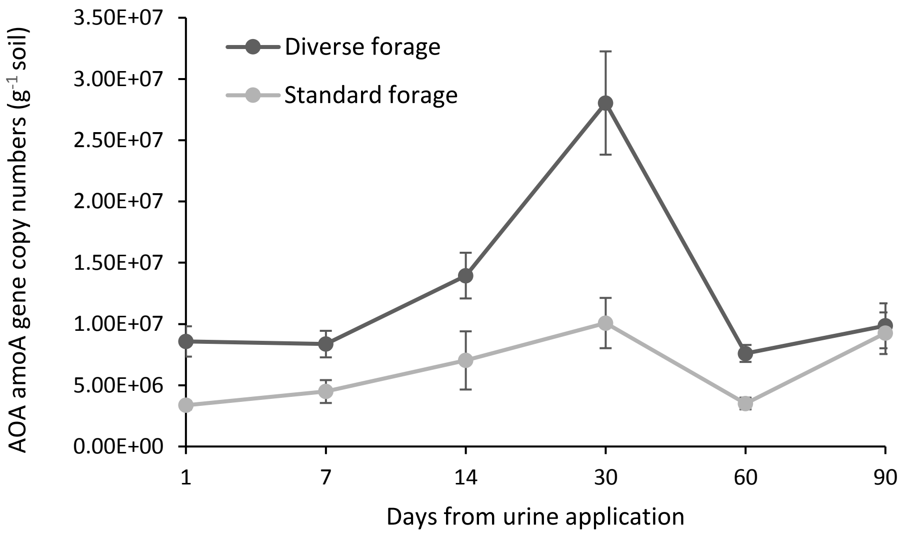


Figure 5.10. Mean AOA *amoA* (copy numbers g^{-1} soil) abundance in the soil as affected by forage type for the urine only treatments. Error bars are ± SEM.

Table 5.7. Soil AOA and AOB *amoA* abundance (copy numbers g^{-1} soil) from day 1 to day 90 for urine only treated soil blocks.

Irrigation	Forage	Treatment	Log$_{10}$ means AOA amoA gene copy numbers g^{-1} soil						AOB amoA gene copy numbers g^{-1} soil					
			1	**7**	**14**	**30**	**60**	**90**	**1**	**7**	**14**	**30**	**60**	**90**
Pivot	Diverse	Urine	6.792	6.935	6.988	7.185	6.768	7.042	7.101	7.507	7.847	8.248	8.213	7.527
	Standard	Urine	6.400	6.577	6.799	6.599	6.452	7.100	7.210	7.611	8.005	8.344	8.182	7.511
Rotorainer	Diverse	Urine	6.734	6.634	7.121	7.391	6.986	7.117	7.030	7.604	7.602	8.062	8.103	7.584
	Standard	Urine	6.533	6.522	6.566	6.097	6.652	6.919	7.057	7.582	7.689	8.164	8.036	7.595
Flood	Diverse	Urine	7.085	7.054	7.168	7.185	6.824	6.524	6.912	7.273	7.540	7.791	8.038	7.502
	Standard	Urine	6.568	6.583	6.720	6.599	6.399	6.503	7.277	7.689	7.856	8.207	8.175	7.651
LSD (5%) for all comparisons			0.286	0.328	0.568	0.442	0.226	0.330	0.410	0.310	0.397	0.229	0.378	0.152
<u>Significance of main effect</u>														
Irrigation			NS	NS	NS	*	*	***	NS	NS	NS	**	NS	NS
Forage			***	**	*	***	***	NS	NS	NS	NS	**	NS	NS
<u>Significance of interaction</u>														
Irrigation × forage			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

5.4 Discussion

5.4.1 Role of AOB and AOA

It is well known that AOB abundance can increase in soils supplemented with high levels of N (Di *et al.*, 2009; Jia & Conrad, 2009; Verhamme *et al.*, 2011; Taylor *et al.*, 2012). This experiment found that AOB were also the main mediators of nitrification. Although both AOA and AOB were detected in large numbers, it was only the AOB abundance that increased in response to urine application. This is in agreement with Di *et al.* (2009) who found that the number of AOB increased 3.2–10.4 fold in response to the application of urinary–N. It was also found that AOA abundance was suppressed when urinary–N was applied. This suggests that AOA and AOB prefer different soil N conditions to grow. Similar results were observed by Di *et al.* (2009), Di *et al.* (2010) and Parfitt *et al.* (2012). For example, Di *et al.* (2010) found that AOB were more abundant in N rich topsoil, whereas AOA were more abundant in the subsoils, thus it was hypothesised that AOA have a preference for low fertility environments. This is supported by the findings of Offre *et al.* (2009) who found that an increase in AOA abundance has been associated with nitrification in soils with a continual supply of NH_4^+ at low concentrations.

5.4.2 Effect of forage type

The results from this experiment showed that AOB abundance (day 30) under the diverse forage was 33% lower than the standard forage following urine application. Consequently, the soil NH_4^+–N concentrations remained higher under the diverse forage while soil NO_3^-–N concentrations were initially lower. It has been suggested that plantain has the ability to release nitrification inhibiting root exudates which has resulted in biological nitrification inhibition (BNI) occurring (Rauber *et al.*, 2008; Dietz *et al.*, 2013; Massaccesi *et al.*, 2015). The results from this experiment support such a hypothesis. Similar trends were observed by Dietz *et al.* (2013) who found that adding fresh plantain residues to soil resulted in lower NO_3^-–N accumulation (days 14–28) and higher NH_4^+–N accumulation (days 1–14) compared to the control treatments. Furthermore, when aucubin, a plant secondary metabolite (PSM) found in plantain was applied to soil, Dietz *et al.* (2013) found that after 28 days, the control treatment (22.86 µg g^{-1} DM soil) showed a significantly higher NO_3^-–N concentration than the aucubin treated soils (10.40 µg g^{-1} DM soil). Simultaneously, the NH_4^+–N concentration was increasing in the aucubin treated soil (8.46 µg g^{-1} DM soil) compared with the control (2.64 µg g^{-1} DM soil), thus indicating some inhibition and nitrification. For a recent review on aucubin and other PSM, the reader is referred to Gardiner *et al.* (2016).

The results from this experiment also show that nitrification inhibition can occur under diverse forages when the forage contains 20–30% plantain. Massaccesi *et al.* (2015) observed significantly lower NO_3^-–N concentrations, and nitrification rates under plantain compared with two other grassland species (*Anthoxanthum odoratum* and *Lotus corniculatus*). Massaccesi *et al.* (2015) also found that as the proportion of plantain in the sward increased, the rates of nitrification decreased. This suggests that the proportion of plantain in a diverse forage is an important factor to consider when evaluating diverse forages as an option to mitigate N loss in grazed forage systems. Additionally, the age of the forage is reported to affect the rate at which nitrification inhibition occurs, with greater nitrification inhibition occurring under older plants (Zakir *et al.*, 2008; Subbarao *et al.*, 2012). The diverse forage used in this experiment had been established for two years at the time of soil block collection. It is therefore possible that as the plants mature, a greater degree of nitrification inhibition may be observed.

Interestingly, the results from this experiment also showed that AOA abundance (days 1, 7, 14, 30 and 60) under the diverse was greater than the standard forage following urine application. As previously, mentioned AOA were not considered the main mediators of nitrification due to the significantly larger response in AOB abundance following urine application. The mechanisms responsible for the greater AOA abundance under the diverse forage in this experiment are uncertain and more research is required to determine the effect diverse forages containing plantain on AOA abundance under cow urine patches.

5.4.3 Mechanisms of biological nitrification inhibition

The mechanisms by which these plants produce chemicals to inhibit nitrification are not well understood. Commercial nitrification inhibitors, such as dicyandiamide (DCD), inhibit the first step of nitrification by binding to the active site and deactivating the ammonia monooxygenase (AMO) enzyme thus slowing the conversion of NH_3 to NH_2OH (Di & Cameron, 2016). As a result, there are higher concentrations of NH_4^+ and lower NO_3^- concentrations present in the soil. Plant produced BNI compounds have been shown to act in a similar way by deactivating the AMO enzyme (McCarty, 1999; Zakir *et al.*, 2008; Subbarao *et al.*, 2009). However, evidence suggests that the plant produced compounds function by blocking both the AMO and hydroxylamino oxidoreductase (HAO) enzymatic pathways (NH_2OH to NO_2^-) (Subbarao *et al.*, 2007b; Subbarao *et al.*, 2009). For example, Brachialactone, a BNI substance identified in the tropical grass species *Brachiaria humidicola,* was shown to inhibit *Nitrosomonas* function by blocking the AMO enzymatic pathway and to a lesser extent the HAO enzymatic pathway (Subbarao *et al.*, 2009). Subbarao *et al.* (2009) also found that when crude root extracts from *Brachiaria humidicola* were used, an inhibitory effect of similar strength occurred between two enzymatic pathways. It was therefore concluded that certain plant species may be capable of releasing multiple BNI substances which have different modes of action. This is thought to be an advantage in reducing vulnerability to genetic change in nitrifier populations. Interestingly, studies also suggest that the presence of NH_4^+ in the root environment is critical for the release of BNI compounds (Subbarao *et al.*, 2007c; Zhu *et al.*, 2012). For example, the release of BNI compounds from *Leymus racemosus* was observed to only be maintained when NH_4^+ was present in the environment (Subbarao *et al.*, 2007b). It is therefore possible that BNI compounds are released as a defence mechanism to protect NH_4^+ from nitrifiers and enhance plant N availability. Currently, BNI activity has mainly been associated with low N environments. Recent work by Byrnes *et al.* (2017), measured greater nitrification, denitrification and AOA abundance suppression from urine patches under the tropical forage grass *Brachiaria humidicola* cv. Tully (high BNI capacity) when compared with the tropical forage grass *Brachiaria hybrid* cv. Mulato (low BNI capacity). As a result, nitrous oxide emissions were lower from urine patches under the grass *Brachiaria humidicola.* The results from this experiment also suggest that certain plant species, such as plantain, are able to produce BNI compounds at a concentration high enough to inhibit microbial activity in relatively high N environments such as the urine patch. However, isolating potential BNI compounds under the diverse forage was outside the scope of this experiment. Further investigation is therefore required to determine the BNI compounds that are produced by plantain and the mechanisms driving the inhibition of nitrification that was observed in this experiment.

5.4.4 Effect of irrigation

The results from this experiment also found that irrigation type had little effect on AOB and AOA abundance under both forage types, and followed no observable trend. The soil NH_4^+–N concentration for all irrigation types peaked on day one (urine treatments) before gradually decreasing, over time while the soil NO_3^-–N concentration peaked between days 14 and 30. It has been established that soil moisture content affects the growth of nitrifiers in the soil (Austin *et al.*, 2004; Sänger *et al.*, 2011; Placella & Firestone, 2013; Di *et al.*, 2014). For example, Di *et al.* (2014) found that when the soil moisture content was at 60% field capacity (FC), the growth of both AOB and AOA was significantly restricted. However, when the soil moisture content increased from 60% FC to either 100% FC or 130% FC, the AOB population abundance increased, particularly in urine treated soil. Variation in soil moisture content following irrigation events could therefore possibly explain why higher AOB abundance was observed under pivot irrigation compared with flood irrigation on day 30. The long return period under flood irrigation (18 days) would cause the soil moisture content to have undergone both dry and saturated periods. Placella and Firestone (2013) reported an incubation study with dry grassland soils where the ammonia oxidizers exhibited rapid responses to water addition with an increase in amoA gene transcripts within 1 h of wetting. It is therefore possible that nitrifier abundance was limited for periods of time under the flood irrigation treatment. Because irrigation was applied as per recommended farm practice (Canterbury region), the small variations observed between the irrigation types may also have been a result of sampling date in relation to when irrigation water was last applied for each irrigation type.

5.5 Conclusions

The main conclusions drawn from this experiment are:

- AOB abundance was lower under the diverse forage containing plantain compared with the standard forage. Consequently, soil NH_4^+–N concentrations remained greater under the diverse forage while the soil NO_3^-–N concentration was lower.

- Lower soil NO_3^-–N concentrations under the diverse forage were attributed to the release of biological nitrification inhibiting (BNI) compounds into the soil by the plantain.

- Irrigation type had little effect on AOB abundance and nitrification rates under diverse and standard forage types.

- These results demonstrate the potential for diverse forages that include plantain to mitigate N loss from cow urine patches, under a range of irrigation types. However, further research is required to determine the mechanisms governing BNI release under plantain rich forages. It would also be beneficial to determine the proportion of plantain required in a diverse forage mix to obtain an optimum level of nitrification inhibition.

Chapter 6

Effect of irrigation type, forage type and urine application date on nitrate leaching losses

6.1 Introduction

6.1.1 Irrigation type

The urine patch represents a major nitrogen (N) loss pathway in New Zealand grazed forage systems (Selbie *et al.*, 2015). Typically, the N deposited in the urine patch exceeds plant nutritional requirements thus surplus N (in the form of nitrate [NO_3^-]) can be leached from the soil profile when drainage occurs (Cameron *et al.*, 2013). Irrigation in New Zealand is rapidly expanding Irrigation New Zealand (2017), and the type of irrigation system used can affect the quantity, intensity and timing that water is applied (Moore, 2002; Aqualinc Reseach Ltd, 2006; Cichota *et al.*, 2016). This can affect water and solute movement down the soil profile and thus the potential for NO_3^- leaching losses to occur (Cameron *et al.*, 2013; Cichota *et al.*, 2016).

Three main types of irrigation are used in New Zealand: (i) pivot/spray, (ii) rotorainer irrigation, and to a lesser extent (iii) flood irrigation (Irrigation New Zealand, 2017). Large irrigation volumes, such as those applied with flood irrigation, typically correspond with a greater leaching depth and increased NO_3^- leaching losses due to the excess water passing through the soil (Di *et al.*, 2002; Moore, 2002; Daudén *et al.*, 2004). Once leached below the root zone, N can no longer be taken up by plants and NO_3^- leaching losses can occur. This can lead to surface and/or ground water contamination (Cameron *et al.*, 2013). A *meta*-analysis by Quemada *et al.* (2013) found that management practices which adjust water application rates to meet crop needs, reduced NO_3^- leaching by 80% without reducing crop yield. Chapter Four of this thesis also demonstrated that when spray irrigation was applied at an 'optimum' rate, NO_3^- leaching losses from spring deposited urine were reduced. However, there is uncertainty about how irrigation type may affect plant N uptake and NO_3^- leaching losses, particularly for diverse forages containing plantain (*Plantago lanceolata*).

Hypothesis: Different irrigation types (pivot, rotorainer or flood) will result in different herbage N uptake, drainage and NO_3^- leaching losses.

Hypothesis: Nitrate leaching losses will be lower from urine deposited during the early summer compared to late summer due to greater opportunity for plant N uptake over the summer period.

6.1.2 Forage type

Recently, there has been increased interest in the incorporation of plantain into diverse forages. Lower urinary–N concentrations have been observed from dairy cows grazing diverse forages containing plantain compared with cows grazing a standard perennial ryegrass (*Lolium perenne*) and white clover forage (*Trifolium repens*) (Woodward *et al.*, 2012; Totty *et al.*, 2013; Edwards *et al.*, 2015; Box *et al.*, 2016). This has resulted in a lower N loading rate in the urine patches deposited by cows grazing a diverse forage and therefore the potential to reduce NO_3^- leaching losses (Selbie *et al.*, 2015). It has also been suggested that diverse forages could be used to mitigate NO_3^- leaching losses through niche separation and N capture (Sanderson *et al.*, 2007), and through the release of biological nitrification inhibiting (BNI) compounds into the soil by plant species such as plantain (Subbarao *et al.*, 2012; Dietz *et al.*, 2013).

Hypothesis: Nitrate leaching losses will be lower under diverse forages containing plantain compared with standard perennial ryegrass and white clover forages due to the release of biological nitrification inhibiting compounds by plantain.

6.1.3 Objectives

The objectives of this experiment were therefore to: (i) quantify the effect of three different irrigation types (pivot, rotorainer and flood) on plant N uptake and subsequent NO_3^- leaching losses from cow urine patches; (ii) determine the effect of forage type on NO_3^- leaching losses from urine patches; and (iii) determine the fate of early summer (December) and late-summer (February) deposited urine.

6.2 Materials and methods

6.2.1 Experiment description, forage type and preparation

Sixty intact soil monolith lysimeters (500 mm diameter × 700 mm depth) were taken from standard and diverse forage field plots located on the Lincoln University Research Dairy Farm (LURDF) and were installed in the LURDF field facility (Figure A.1). The lysimeters were collected using established protocols outlined by Cameron *et al.* (1992) (Plate 6.1). A detailed description of the lysimeter collection and installation process is described in Section 3.4.

Plate 6.1. Chapter Six lysimeters which were ready to be lifted out and processed (left) and fully installed lysimeters in the trench facility (right).

A set of 30 lysimeters received a cow urine application in December (early summer). A second set of 30 lysimeters and 24 soil blocks received a cow urine application in February (late summer).

The forages from both lysimeter collection sites were sown in March 2014 and at the time of extraction were two years old. The standard forage contained perennial ryegrass and white clover. The diverse forage contained perennial ryegrass, white clover and plantain. Sowing rates and cultivars are shown in Table 6.1. The perennial ryegrass cultivar used was 'Trojan' with NEA2 endophyte. Trojan is a late heading and high yielding diploid cultivar with high production on the shoulders of the season (winter/early spring and autumn) (Agriseeds Ltd, 2013).

Table 6.1. Species, cultivars and sowing rates for standard and diverse forages.

Plant species	Scientific name	Cultivar	Sowing rate (kg seeds ha^{-1}) Standard forage	Sowing rate (kg seeds ha^{-1}) Diverse forage
Perennial ryegrass	*Lolium perenne* L.	Trojan	20	20
White clover	*Trifolium repens* L.	Kopu ll	3	3
Plantain	*Plantago lanceolata* L.	Tonic	0	3

Visual observations in the field, and photographs of individual lysimeters and soil blocks were used to estimate the proportion of plantain in the diverse forage. It was estimated that the proportion of plantain in the diverse forage ranged from 20-30% (Plate 6.2).

Plate 6.2. Diverse forage lysimeter (top left) and soil block (top right) containing perennial ryegrass, white clover and plantain. Diverse forage site prior to lysimeter collection (bottom left) and a standard forage lysimeter containing perennial ryegrass and white clover (bottom right).

6.2.2 Experimental design

The experimental design consisted of twelve treatments including three irrigation types (pivot vs. rotorainer vs. flood), two forage types (standard vs. diverse), two urine application dates (December vs. February) and one urinary–N application rate (700 kg N ha^{-1}) (Table 6.2). The treatments were replicated five times and were arranged in a randomised split-split plot design. Dairy cow urine was applied to one set of lysimeters on the 10 December 2015 and to another set of lysimeters on the 19 February 2016, and measurements were taken thereafter until the 30 September 2016.

Table 6.2. Description of lysimeter treatments.

Treatment no.	Irrigation type	Irrigation rate (mm)	Irrigation frequency interval (days)	Forage type	Urine application date	Urine treatment (kg N ha^{-1})
1	Pivot	15	3	Standard	February	700
2	Pivot	15	3	Diverse	February	700
3	Pivot	15	3	Standard	December	700
4	Pivot	15	3	Diverse	December	700
5	Rotorainer	45	9	Standard	February	700
6	Rotorainer	45	9	Diverse	February	700
7	Rotorainer	45	9	Standard	December	700
8	Rotorainer	45	9	Diverse	December	700
9	Flood	90	18	Standard	February	700
10	Flood	90	18	Diverse	February	700
11	Flood	90	18	Standard	December	700
12	Flood	90	18	Diverse	December	700

6.2.3 Urine collection and application

On the 9 December 2015 (early summer), fresh dairy cow urine was collected from Friesian × Jersey cross cows on the Lincoln University Dairy Farm (LUDF) during the afternoon milking. The total urinary–N concentration was 3.7 g N L^{-1}. This was standardised to a concentration of 7 g N L^{-1} using urea, and glycine in a 9:1 ratio (Bathurst, 1952). A bromide (Br^-) tracer, in the form of potassium bromide (KBr), was also dissolved in the urine at a rate equivalent to 50 kg Br^- ha^{-1}. The Br^- tracer was added to the urine to measure the leaching breakthrough curve for a non-biologically active tracer. Individual lysimeters (December application) received a 2 L surface application of urine to simulate urine patch deposition by grazing dairy cows at a loading rate equivalent to 700 kg N ha^{-1} (Selbie *et al.*, 2015). The lysimeters not receiving urine (February application) had 2 L of water applied to them to maintain a similar soil moisture content between treatments. Following the same procedure, fresh dairy cow urine was again collected from LUDF on the 18 February 2016 (late summer). The total urinary–N concentration was 5.2 g N L^{-1}, which was again standardised to a concentration of 7 g N L^{-1}, and a Br^- tracer was again dissolved in the urine at a rate of 50 kg Br^- ha^{-1}. Individual lysimeters (February application) then received a 2 L surface application of urine at a loading rate equivalent to 700 kg N ha^{-1}. Again, 2 L of water was applied to the lysimeters not receiving a urine application.

6.2.4 Irrigation scheduling

From December to April (summer) pivot and rotorainer irrigation treatments were simulated using the irrigation system described in Section 3.7. Flood irrigation was manually applied to individual lysimeters during this period. This involved measuring out 18 L volumes of water (equivalent to 90 mm) and pouring the water onto the lysimeter to simulate flood irrigation. A stainless steel ring was attached to the top of each lysimeter to allow the large volume of water to be applied, and to prevent water overflowing from the lysimeter (Plate 6.3). Each ring was inserted 100 mm into the soil and sealed around the top edge of the lysimeter using silicone sealant. The rings were attached to all the lysimeters to maintain consistent micro environmental conditions between treatments. The pivot irrigation treatment comprised of irrigation every three days at an application rate of 15 mm and at an intensity of 20 mm per hour (Table 6.2). The rotorainer irrigation treatment comprised of irrigation every nine days at an application rate of 45 mm and at an intensity of 20 mm per hour, and the flood irrigation treatment comprised of irrigation every 18 days at an application rate of 90 mm and at an intensity of 90 mm per hour (Table 6.2). Irrigation was applied to match typical irrigation rates for pivot, rotorainer and flood irrigation in the Canterbury region (Hydroservices Ltd, 2014). The soil moisture sensors described in Section 3.6.3 were used to monitor the soil moisture content and adjust irrigation applications when necessary. In the event of natural rainfall, the period of time between irrigation events was extended to remain consistent with typical irrigation practice.

Plate 6.3. Stainless steel ring attached to the lysimeter for flood irrigation (left), and a lysimeter after a flood irrigation event (right).

6.2.5 Fertiliser

Prior to treatment application, all lysimeters received a maintenance fertiliser application in the form of 20% potash super sulphur (6.4–10–16–14) equivalent to 65 kg P ha^{-1} (standard forage) and 50 kg P ha^{-1} (diverse forage). The rate of fertiliser applied was determined from soil test results (Table 3.2). To simulate typical farm management practices and paddock conditions, urea fertiliser was applied in split applications to provide an annual rate of 125 kg N ha^{-1}. As per recommended farm practice, N fertiliser was applied between October and April in split applications of 25 kg N ha^{-1} (Fertiliser Assosiation, 2009). All fertiliser was hand applied evenly across the surface of the lysimeter. This was followed with 10 mm of irrigation to wash the fertiliser into the soil and to prevent volatilisation.

6.2.6 Root distribution and soil sampling

On the 30 September 2016, all lysimeters were destructively sampled to determine the plant root distribution, and the soil inorganic N and total N content. Using an auger, soil cores were taken down the soil profile at depths of 0–100 mm, 100–200 mm, 200–400 mm and 400–600 mm (Plate 6.4). Three samples were taken at each depth, one for root distribution and two (combined) for soil analysis. All samples were refrigerated at 4°C prior to processing.

Root samples were then hand washed on a sieve to separate and remove the soil from the plant roots (Plate 6.4). This involved placing the soil sample in a fine mesh tray and carefully washing the soil off the plant roots using the water jets from a hand-held hose. Once the soil was removed, the root sample was then placed in a clean bag and refrigerated at 4°C until analysed.

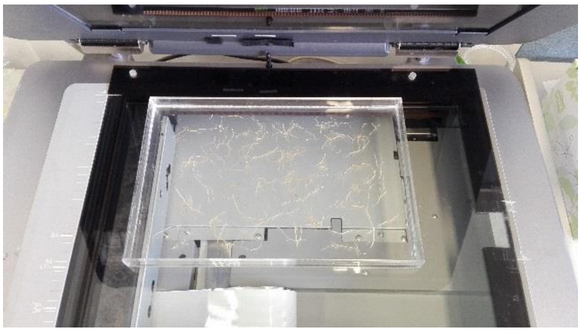
Root measurements were determined using the computer software WinRHIZO (Reg V2009c; Regent Instruments Inc., Quebec City, Canada), an interactive scanner based image analysis system that controls scanning, digitalising, and analysis of root samples. The root samples were floated in clear plastic trays using water to untangle the roots and minimise overlapping (Plate 6.4). The water that was used was previously boiled, then cooled, to remove any oxygen bubbles. This ensured a clear image was obtained. The root samples were then scanned and digitalised by creating grey-scale images (400 dpi, with a transmitted light unit (TLU), EPSON EXPRESSION 10000XL 3.49) (Plate 6.4). Any root overlap was detected and corrected for in the WinRHIZO program. Measurements included total root length, average root diameter, total surface area and total root volume.

(a) Destructive soil sampling using an auger.

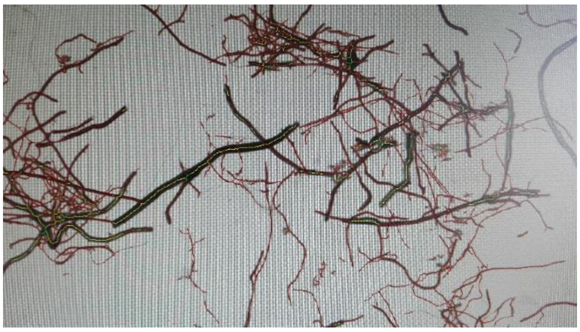
(b) A lysimeter after soil cores were taken at varying depths down the soil profile.

(c) Soil being removed from the plant roots using a hose and fine mesh tray.

(d) Plant roots after the cleaning process.



(e) Plant roots spread out for scanning using the WinRhizo root scanning machine.



(f) Computer image of scanned roots following the root measurement analysis.

Plate 6.4. Destructive sampling and processing of lysimeters for root distribution, and soil analysis (a) to (f).

6.2.7 Analysis

Leachate and herbage sub-samples were collected from individual lysimeters for laboratory analysis. Sampling methods are outlined in Section 3.6.

6.2.7.1 Leachate

Ammonium and nitrate

The analysis of NH_4^+–N and NO_3^-–N concentrations in the leachate was conducted using a Foss FIAstar 5000 twin channel analyser (Foss Tecator AB, Hoganas, Sweden) (Gal *et al.*, 2004) as is outlined in Section 4.2.6.1.

Bromide

Leachate samples were analysed for Br^- concentration using a Dionex DX-2100 ion exchange chromatography system (Dionex Corporation, California. USA). The system was suppressed with an Anion Self Regeneration Suppressor (Dionex AERS 500) and detection was through conductivity. Samples were filtered through a 2 µm membrane filter prior to analysis and were separated with a weak sodium carbonate/sodium bicarbonate eluent on a Dionex As9-SC column.

6.2.7.2 Herbage

Total nitrogen

The analysis of herbage total N was conducted using an Elementar Vario-Max CN Elementar Analyser (Elementar GmbH, Hanau, Germany) as is outlined in Section 4.2.6.2.

6.2.7.3 Soil

Inorganic nitrogen

Potassium chloride (KCl) extracts were carried out on 5 g of field moist soil using 25 mL of 2M KCl extraction solution (Keeney & Nelson, 1982). The soil samples were thoroughly mixed prior to analysis, and a sub-sample was oven-dried at 105°C to enable correction for soil moisture content. The KCl extract and soil suspension was shaken for 1 hour on an end over shaker and then centrifuged at 4000 rpm for 10 minutes. Once removed, the sample was filtered through a 110 mm Avantec 5C filter paper funnel, and frozen at -20°C until analysed (Section 4.2.6.1). Soil inorganic N concentrations (NO_3^- and NH_4^+) were determined using a FOSS FIAstar 5000 twin channel analyser (Foss Tecator AB, Hoganas, Sweden).

Total soil nitrogen

Total soil N was determined using an Elementar Vario-Max CN Elementar Analyser (Elementar GmbH, Hanau, Germany). The soil samples were thoroughly mixed and a sub-sample was oven-dried at 60°C for 24 hours. The dried soil samples were then finely ground for analysis (see Section 4.2.6.2).

Soil moisture

A sub-sample was taken to measure the moisture content of each soil sample. Approximately 10 g of soil was taken, oven-dried at 105°C for 24 hours, and reweighed. The soil moisture was determined using Equation 8.

$$\text{Soil moisture (\%)} = ((\text{wet soil (g)} - \text{dry soil (g)}) \times 100)/\text{dry soil (g)} \tag{8}$$

6.2.8 Statistical analysis

Statistical analysis was carried out using analysis of variance (ANOVA) (Genstat 16th Edition, VSN International Ltd). Standard errors of the mean were calculated and presented with the mean values or the LSD ($P < 0.05$) was calculated from the means. The total drainage, total NH_4^+ and NO_3^-, and total herbage DM yield and N uptake data were log-transformed to normalise the variance and to determine statistical treatment effects.

6.3 Results

6.3.1 Temperature

Daily air and ground temperatures are given in Figure 6.1. The minimum air and ground temperatures were recorded on 11 July 2016 and 8 August 2016 at 1.6°C and 4.1°C, respectively. The maximum air and ground temperatures were measured on 26 February 2016 and 17 February 2016 at 25°C and 22°C, respectively.

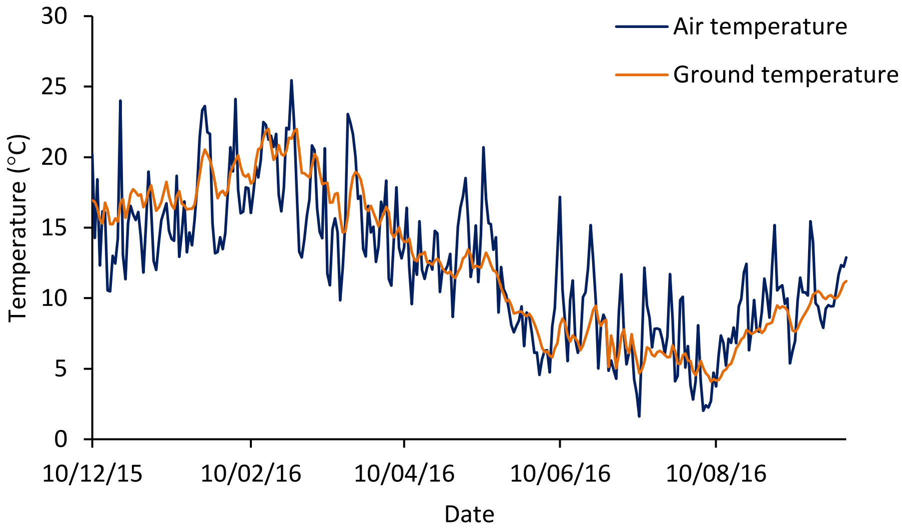


Figure 6.1. Average daily air and ground temperature from December 2015 to September 2016.

6.3.2 Water inputs

From December 2015 to April 2016, the total amounts of irrigation applied were 422 mm under the pivot irrigation treatment (Figure 6.2A), 468 mm under the rotorainer irrigation treatment (Figure 6.2B), and 555 mm under the flood irrigation treatment (Figure 6.2C). The total rainfall (natural and simulated) for the experimental period was 666 mm, the majority of this fell in May, August and September 2016 (Figure 6.2).

As described in Section 6.2.4, each irrigation type was scheduled according to the amount of rainfall received (as would occur on farm with best practice irrigation management). To achieve this a model which accumulated daily climatic values as reference point was used to schedule irrigation events as described in Section 3.7.

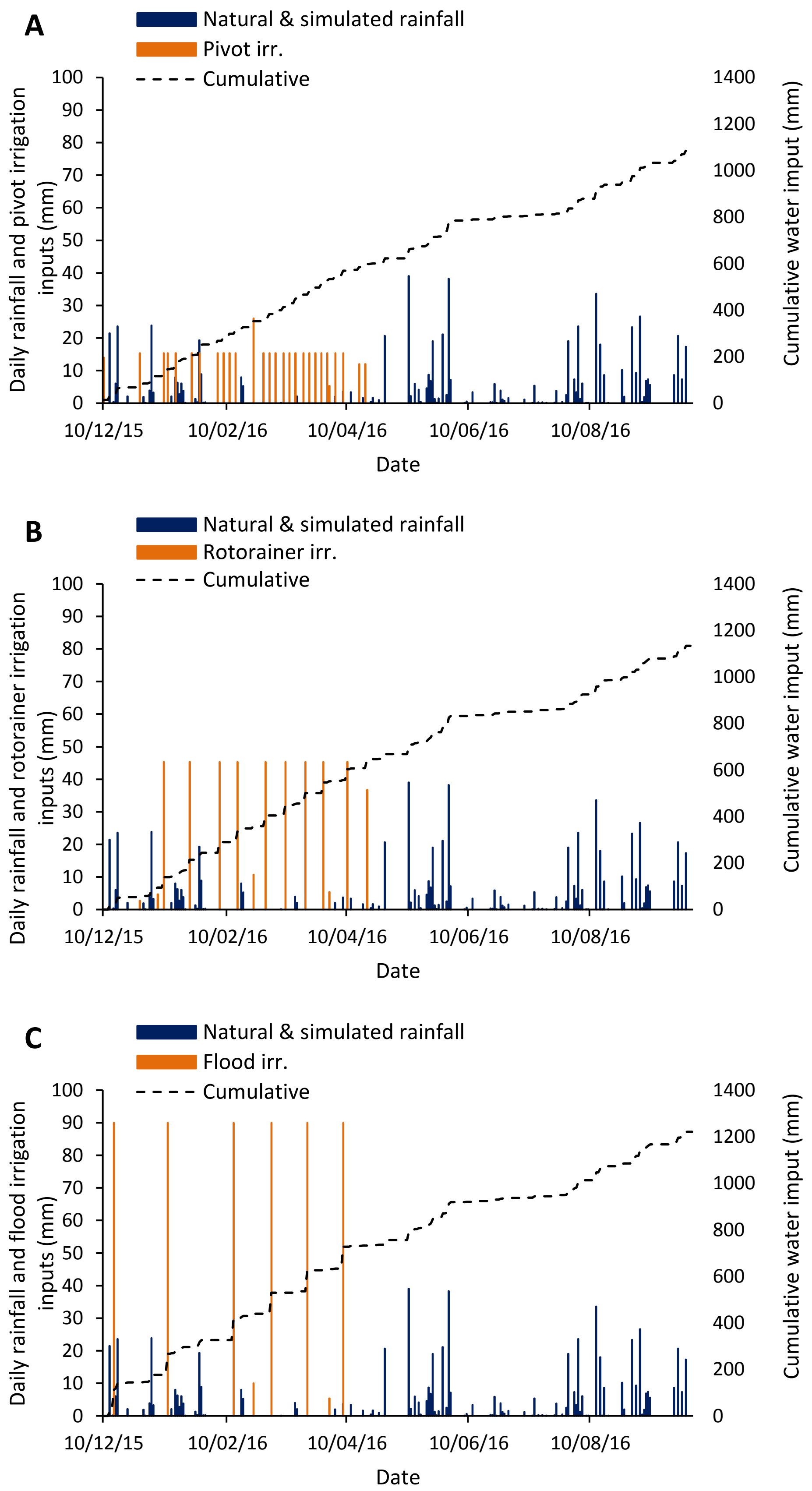


Figure 6.2. Cumulative and daily water inputs (mm) from December 2015 to September 2016 for pivot irrigation (A), rotorainer irrigation (B) and flood irrigation (C).

6.3.3 Soil moisture content

During the irrigation season (December 2015 to April 2016), the three irrigation treatments produced different fluctuations, in the soil moisture content, in the top 0–200 mm of the lysimeter (Figure 6.3). The flood and rotorainer irrigation produced large spikes in the soil moisture content (Figure 6.3B&C). In contrast, the pivot irrigation maintained a relatively constant soil moisture content over the summer period (Figure 6.3A). From May 2016 onwards, the soil moisture content followed a similar trend for all irrigation treatments.

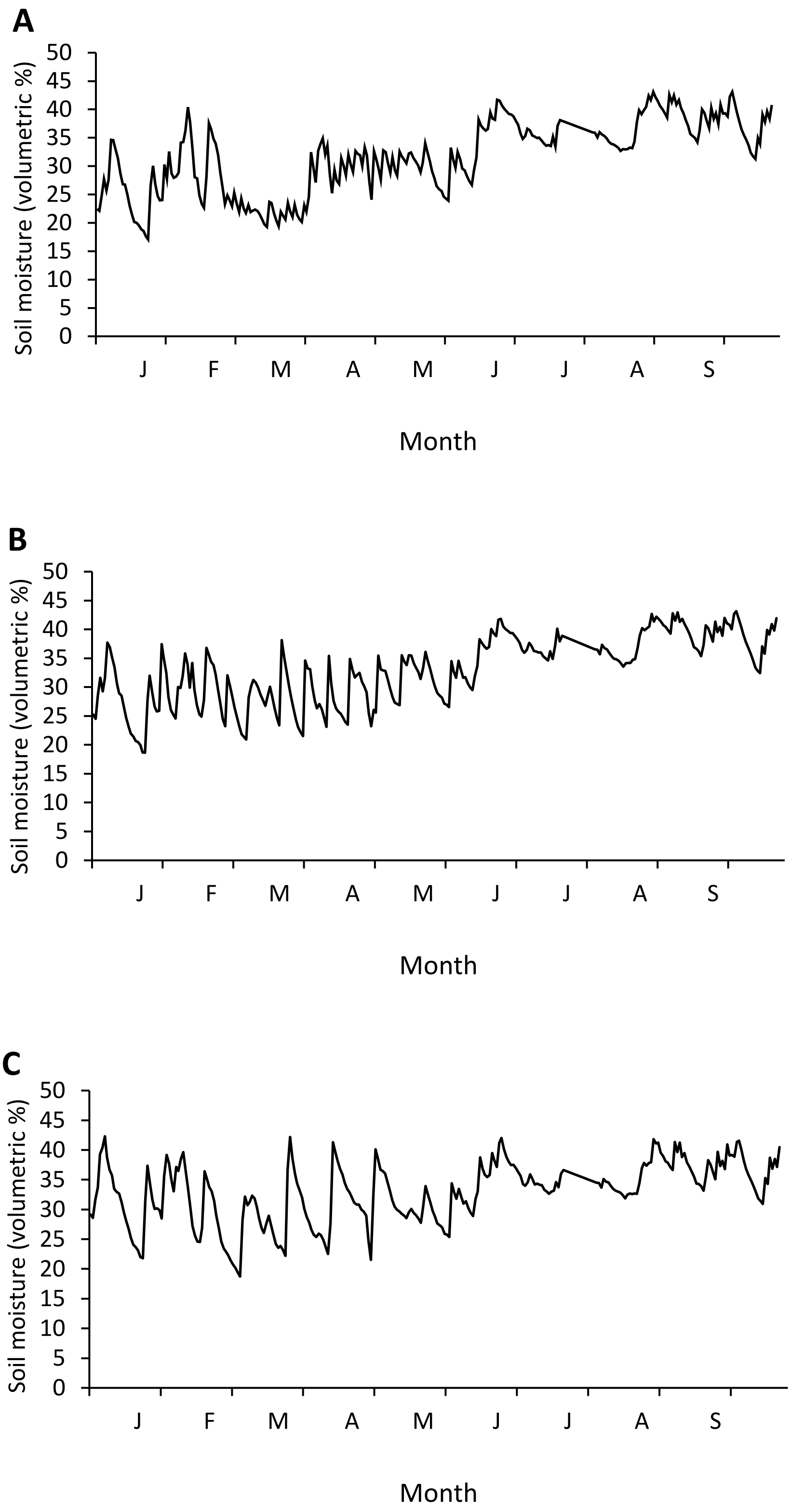


Figure 6.3. Soil moisture (volumetric %) at 0–200 mm for lysimeters receiving pivot (A), rotorainer (B) or flood (C) irrigation.

6.3.4 Drainage

The amount of drainage over the summer period was low under pivot and rotorainer irrigation (Figure 6.4). The greatest amount of drainage was recorded during the autumn and winter period for all irrigation treatments (Figure 6.4). Due to a lack of rainfall, no drainage was collected during July 2016.

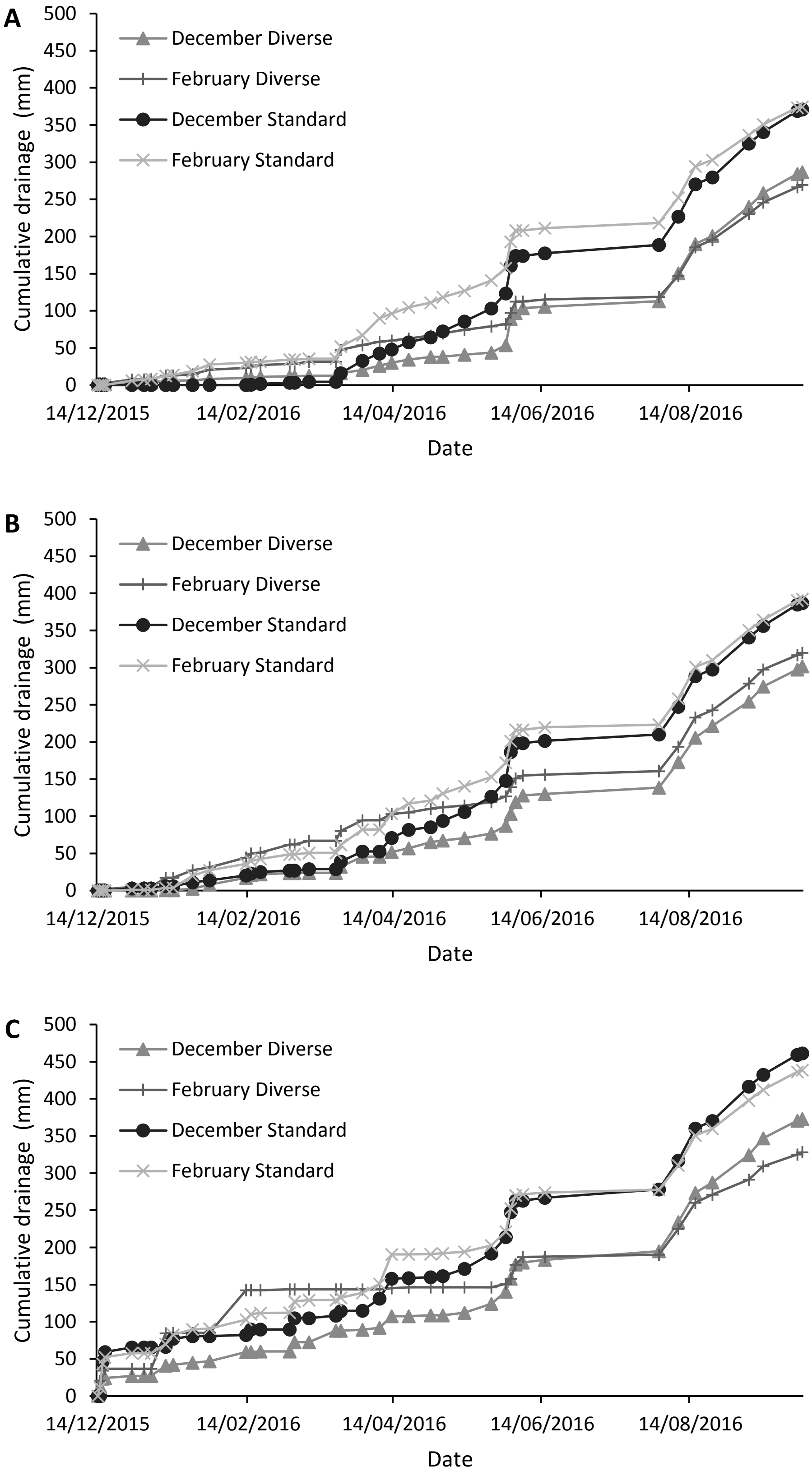


Figure 6.4. Cumulative drainage (mm) as affected by pivot irrigation (A), rotorainer irrigation (B) and flood irrigation (C).

Averaged across all treatments, the amount of drainage was significantly affected by forage type ($P < 0.001$) (Table 6.3). The amount of drainage from the diverse forage was 21% less than the standard forage when urine was applied in December, and 28% less when urine was applied in February ($P < 0.05$). The total amount of drainage was not significantly affected by irrigation type ($P = 0.338$) (Table 6.3).

December urine application

For the December urine application treatments, the greatest amount of drainage occurred from the flood irrigation treatments, with 461 mm of drainage under the standard forage and 373 mm of drainage under the diverse forage (Figure 6.5A). Under the rotorainer irrigation treatment, the amount of drainage from the standard forage (387 mm) was significantly greater ($P < 0.05$) than the amount of drainage from the diverse forage (302 mm) (Figure 6.5A). There was no significant difference in the amount of drainage that occurred between irrigation treatments.

February urine application

For the February urine application treatments, the greatest amount of drainage occurred from the flood irrigation treatments, with 439 mm of drainage under the standard forage and 328 mm of drainage under the diverse forage (Figure 6.5B). The amount of drainage under the standard forage was significantly greater ($P < 0.05$) than the amount of drainage under the diverse forage for pivot (46%), rotorainer (35%) and flood (34%) irrigation treatments (Figure 6.5B). There was no significant difference in the amount of drainage that occurred between irrigation treatments.

Table 6.3. Total amount of drainage (mm) from lysimeters as affected by irrigation type, forage type and urine application month.

Irrigation type	Forage type	Urine application month	Log_{10} means drainage (mm)
Pivot	Diverse	December	2.453
	Standard	December	2.522
	Diverse	February	2.412
	Standard	February	2.537
Rotorainer	Diverse	December	2.457
	Standard	December	2.569
	Diverse	February	2.463
	Standard	February	2.572
Flood	Diverse	December	2.566
	Standard	December	2.660
	Diverse	February	2.520
	Standard	February	2.641
LSD (5%) within irrigation regimes			0.096
LSD (5%) for all other comparisons			0.185
<u>Significance of main effect</u>			
Irrigation			NS
Forage			***
Urine app. month			NS
<u>Significance of interaction</u>			
Irrigation × forage			NS
Irrigation × urine app. month			NS
Forage × urine app. month			NS
Irrigation × forage × urine app. month			NS

NS = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

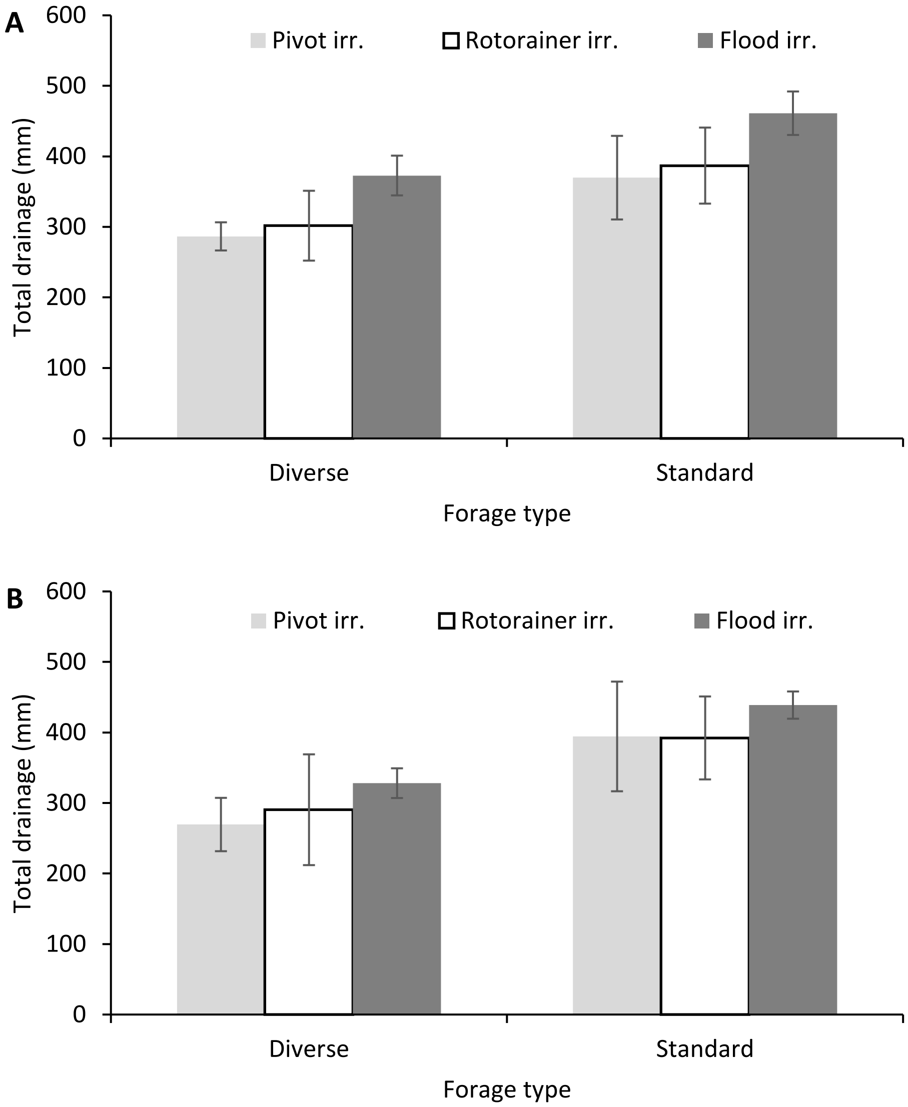


Figure 6.5. Total amount of drainage (mm) averaged from each treatment following urine application in December (A) and February (B). Error bars are ± SEM.

6.3.5 Nitrate concentration in drainage

December urine application

Following the December urine application, the peak NO_3^-–N concentrations in drainage water occurred under the standard forage at 62 mg NO_3^-–N L^{-1} (pivot irrigation), 62 mg NO_3^-–N L^{-1} (rotorainer irrigation) and 40 mg NO_3^-–N L^{-1} (flood irrigation) (Figure 6.6). The peak NO_3^-–N concentrations in drainage water under the diverse forage remained below 16 mg NO_3^-–N L^{-1} for all irrigation treatments (Figure 6.6). The NO_3^-–N concentrations for all treatments peaked before 150 mm of drainage had occurred. The NO_3^-–N concentrations returned to background levels by 250 mm of drainage (Figure 6.6).

February urine application

Following the February urine application, the peak NO_3^-–N concentrations in drainage water occurred under the standard forage at 78 mg NO_3^-–N L^{-1} (pivot irrigation), 52 mg NO_3^-–N L^{-1} (rotorainer irrigation) and 61 mg NO_3^-–N L^{-1} (flood irrigation) (Figure 6.7). The peak NO_3^-–N concentrations in drainage water occurred under the diverse at 16 mg NO_3^-–N L^{-1} (pivot irrigation), 27 mg NO_3^-–N L^{-1} (rotorainer irrigation) and 162 mg NO_3^-–N L^{-1} (flood irrigation) (Figure 6.7). The NO_3^-–N concentrations for all treatments peaked when the amount of drainage was between 50 mm and 250 mm (Figure 6.7).

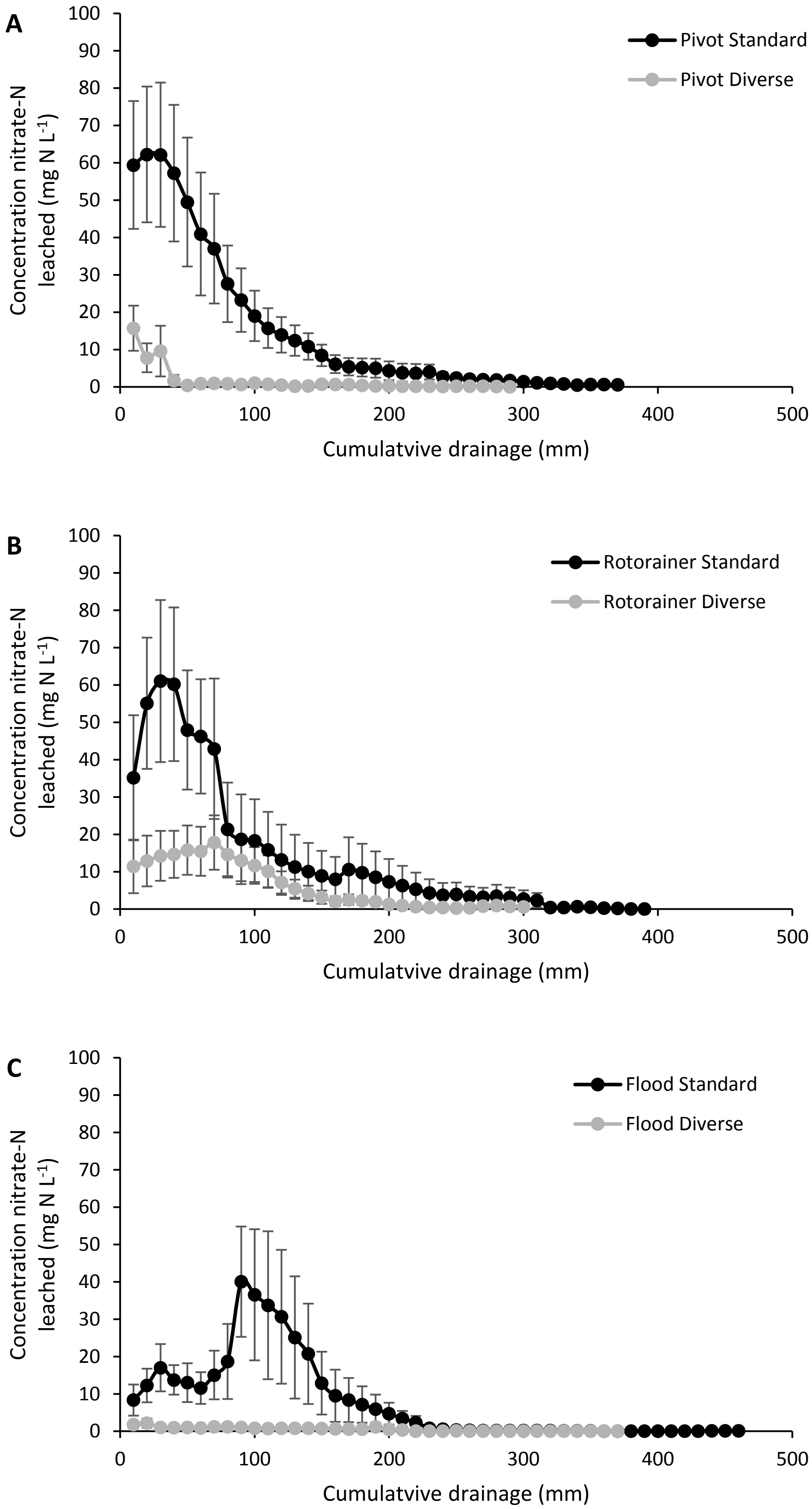


Figure 6.6. Concentration of NO_3^-–N (mg L^{-1}) in drainage following a December urine application as affected by pivot irrigation (A), rotorainer irrigation (B) and flood irrigation (C). Errors bars are ± SEM.

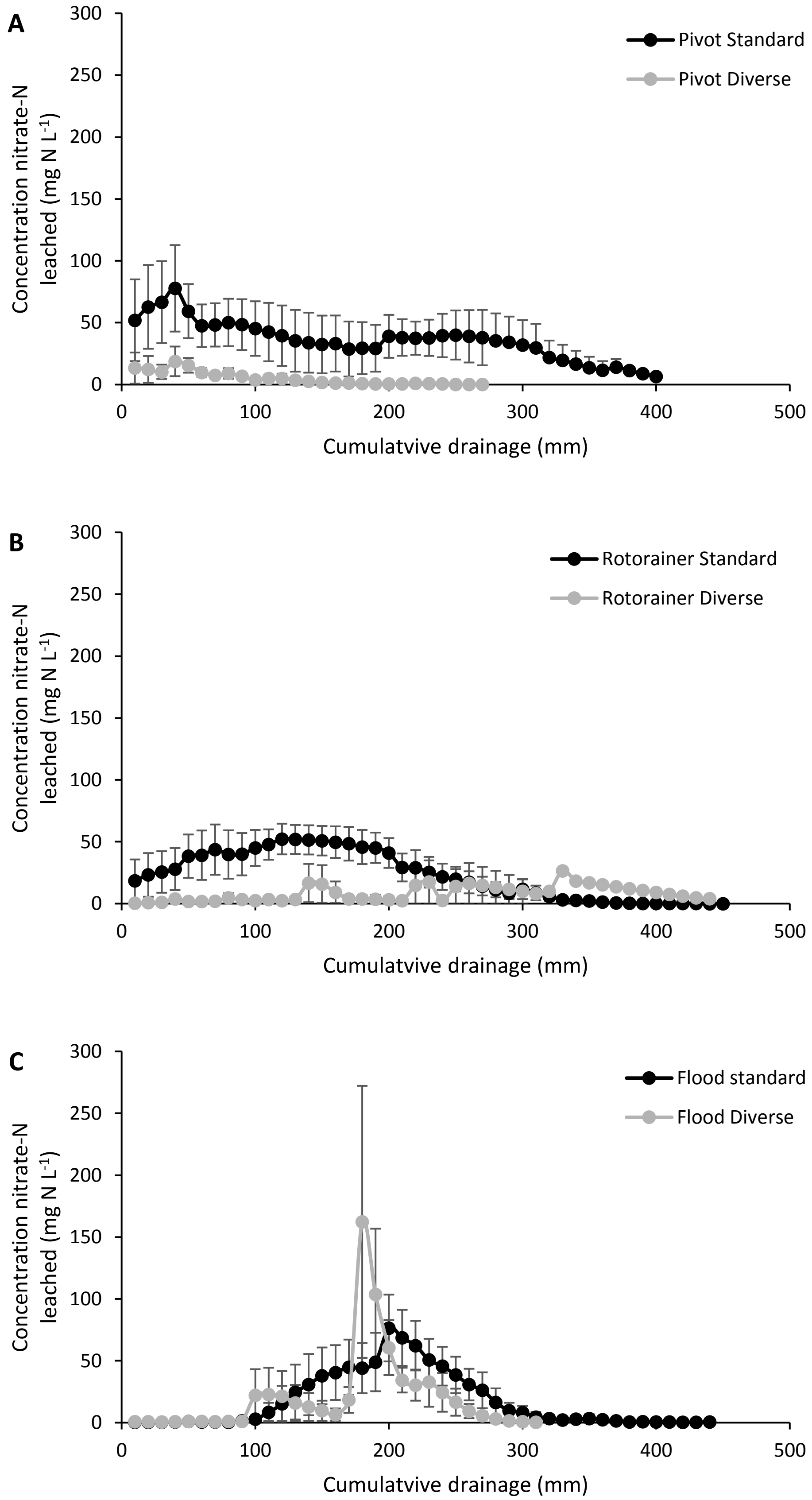


Figure 6.7. Concentration of NO_3^-–N (mg L^{-1}) in drainage following a February urine application as affected by pivot irrigation (A), rotorainer irrigation (B) and flood irrigation (C). Errors bars are ± SEM.

6.3.6 Total nitrate and ammonium leaching losses

Averaged across all treatments, forage type had a highly significant effect ($P < 0.001$) on NO_3^-–N leaching losses (Table 6.4). The NO_3^-–N leaching losses from the diverse forage were 82% less than the standard forage when urine was applied in December and 74% less when urine was applied in February ($P < 0.05$). Averaged across all treatments, NO_3^-–N leaching losses were significantly ($P = 0.002$) affected by urine application month (Table 6.4). The NO_3^-–N leaching losses from urine applied in December were 69% (diverse forage) less than NO_3^-–N leaching losses from urine applied in February ($P < 0.05$). The different irrigation types had no significant ($P = 0.338$) effect on NO_3^-–N leaching losses.

December urine application

Following the December urine application, the greatest amount of NO_3^-–N was leached from the standard forage, and ranged from 37–50 kg NO_3^-–N ha^{-1} under the three irrigation treatments. Nitrate leaching losses from the diverse forage were below 20 kg NO_3^-–N ha^{-1} for all irrigation treatments (Figure 6.8A). Nitrate leaching losses from the diverse forage treatment were significantly ($P < 0.05$) less than from the standard forage treatment. The NO_3^-–N leaching losses from the diverse forage were 92% (flood irrigation), 67% (rotorainer irrigation) and 93% (pivot irrigation) less than the NO_3^-–N leaching losses from the standard forage (Figure 6.8A).

Ammonium leaching losses were below 8 kg NH_4^+–N for all irrigation treatments (Figure 6.9A). The percentage of mineral N as NH_4^+–N ranged from 3.4–74.4% (diverse forage) and 0.7–5.5% (standard forage).

February urine application

Following the February urine application, the greatest amount of NO_3^-–N was leached from the standard forage, and ranged from 69–126 kg NO_3^-–N ha^{-1} under the three irrigation treatments. Nitrate leaching losses from the diverse forage ranged from 10–41 kg NO_3^-–N ha^{-1} under the three irrigation treatments (Figure 6.8B). Nitrate leaching losses from the diverse forage treatment were significantly ($P < 0.05$) less than the standard forage treatment. The NO_3^-–N leaching losses from the diverse forage were 40% (flood irrigation), 67% (rotorainer irrigation) and 92% (pivot irrigation) less than the NO_3^-–N leaching losses from the standard forage (Figure 6.8B).

Ammonium leaching losses were below 9 kg NH_4^+–N for all irrigation treatments (Figure 6.9B). The percentage of mineral N as NH_4^+–N ranged from 4.4–24.1% (diverse forage) and 3.5–3.8% (standard forage).

Table 6.4. Total amount of NO_3^-–N and NH_4^+–N leached (kg ha^{-1}) from lysimeters as affected by irrigation type, forage type and urine application month.

Irrigation type	Forage type	Urine app. month	Log$_{10}$ means: Total NO_3^- loss (kg NO_3^-–N ha^{-1})	Total NH_4^+ loss (kg NH_4^+–N ha^{-1})
Pivot	Diverse	December	0.307	0.218
	Standard	December	1.610	-0.529
	Diverse	February	0.947	0.319
	Standard	February	2.148	-0.009
Rotorainer	Diverse	December	0.534	-0.503
	Standard	December	1.407	-0.100
	Diverse	February	0.633	0.245
	Standard	February	1.953	-0.189
Flood	Diverse	December	0.413	0.118
	Standard	December	1.396	0.376
	Diverse	February	1.736	0.984
	Standard	February	1.617	-0.049
LSD (5%) within irrigation regimes			0.834	0.929
LSD (5%) for all other comparisons			0.827	0.868
Significance of main effect				
Irrigation			NS	NS
Forage			***	NS
Urine app. month			**	NS
Significance of interaction				
Irrigation × forage			NS	NS
Irrigation × urine app. month			NS	NS
Forage × urine app. month			NS	NS
Irrigation × forage × urine app. month			NS	NS

NS = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

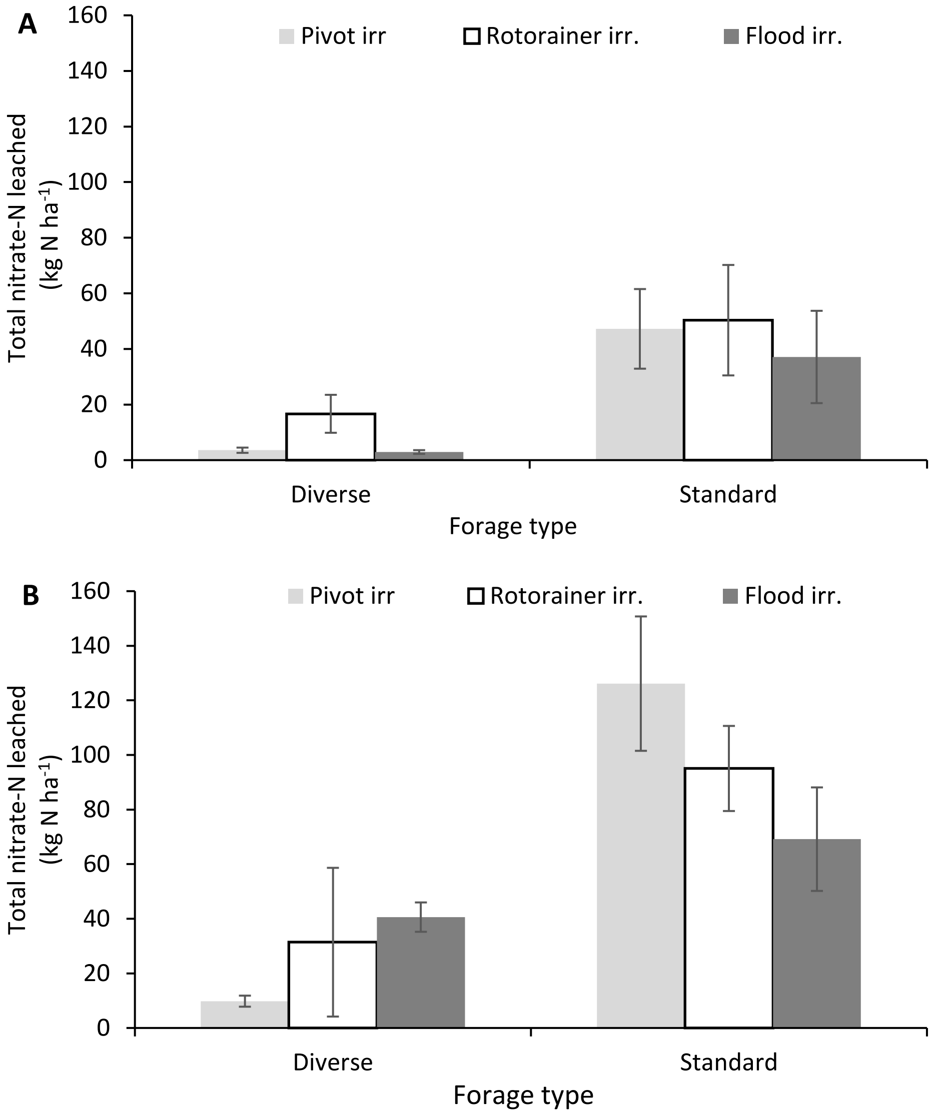


Figure 6.8. Total NO_3^-–N leached (kg ha^{-1}) from lysimeters as affected by irrigation (pivot vs. rotorainer vs. flood), forage type (standard vs. diverse) and urine application date (December (A) and February (B)). Error bars are ± SEM.

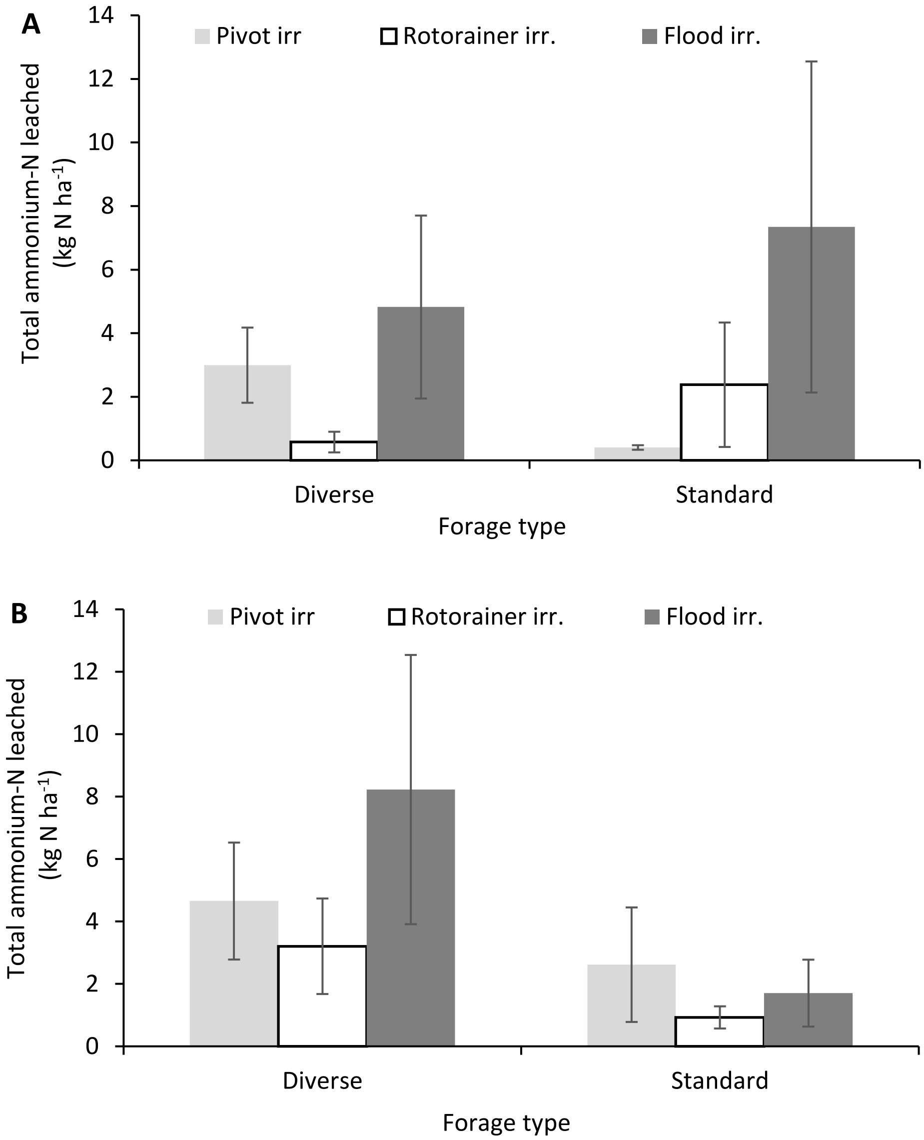


Figure 6.9. Total NH_4^+–N leached (kg ha^{-1}) from lysimeters as affected by irrigation (pivot vs. rotorainer vs. flood), forage type (standard vs. diverse) and urine application date (December (A) and February (B)). Error bars are ± SEM.

6.3.7 Bromide tracer

Averaged across all treatments, bromide recovery in the leachate was significantly affected by forage type ($P < 0.001$) and urine application month ($P = 0.005$). There was a significant interaction between irrigation and forage type ($P < 0.001$), and irrigation, forage type and urine application date ($P < 0.001$). The total amount of Br^- recovered in the leachate is given in Table 6.5. The percentage of Br^- recovered ranged from 16–54%, assuming that the Br^- leached was entirely derived from the Br^- applied in the treatments. The concentration of Br^- in drainage water followed a similar pattern to the NO_3^-–N leaching breakthrough curves following a December and February urine application.

December urine application

Following the December urine application, the peak Br^- concentrations in drainage water occurred under the standard forage at 16 mg Br^- L^{-1} (pivot irrigation), 19 mg Br^- L^{-1} (rotorainer irrigation) and 13 mg Br^- L^{-1} (flood irrigation) (Figure 6.10). The peak Br^- concentrations in drainage water under the diverse forage remained below 10 mg Br^- L^{-1} for all irrigation treatments (Figure 6.10).

February urine application

Following the February urine application, the peak Br^- concentrations in drainage water occurred under the standard forage at 29 mg Br^- L^{-1} (pivot irrigation), 10 mg Br^- L^{-1} (rotorainer irrigation) and 14 mg Br^- L^{-1} (flood irrigation) (Figure 6.11). The peak Br^- concentrations in drainage water occurred under the diverse forage at 8 mg Br^- L^{-1} (pivot irrigation), 6 mg Br^- L^{-1} (rotorainer irrigation) and 44 mg Br^- L^{-1} (flood irrigation) (Figure 6.11).

Table 6.5. Recovery of bromide (kg ha^{-1}) as affected by irrigation (pivot vs. rotorainer vs. flood), forage type (standard vs. diverse) and urine application date (December vs. February).

Urine app. month	Irrigation	Forage	Mean bromide recovery (kg ha^{-1})	Recovery %
December	Pivot	Standard	18 (± 3.7)	36
		Diverse	08 (± 4.0)	17
	Rotorainer	Standard	17 (± 3.6)	34
		Diverse	13 (± 7.0)	26
	Flood	Standard	23 (± 4.0)	46
		Diverse	16 (± 4.8)	31
February	Pivot	Standard	27 (± 5.1)	54
		Diverse	08 (± 3.0)	16
	Rotorainer	Standard	23 (± 6.2)	47
		Diverse	10 (± 9.6)	19
	Flood	Standard	22 (± 1.9)	43
		Diverse	21 (± 2.3)	41

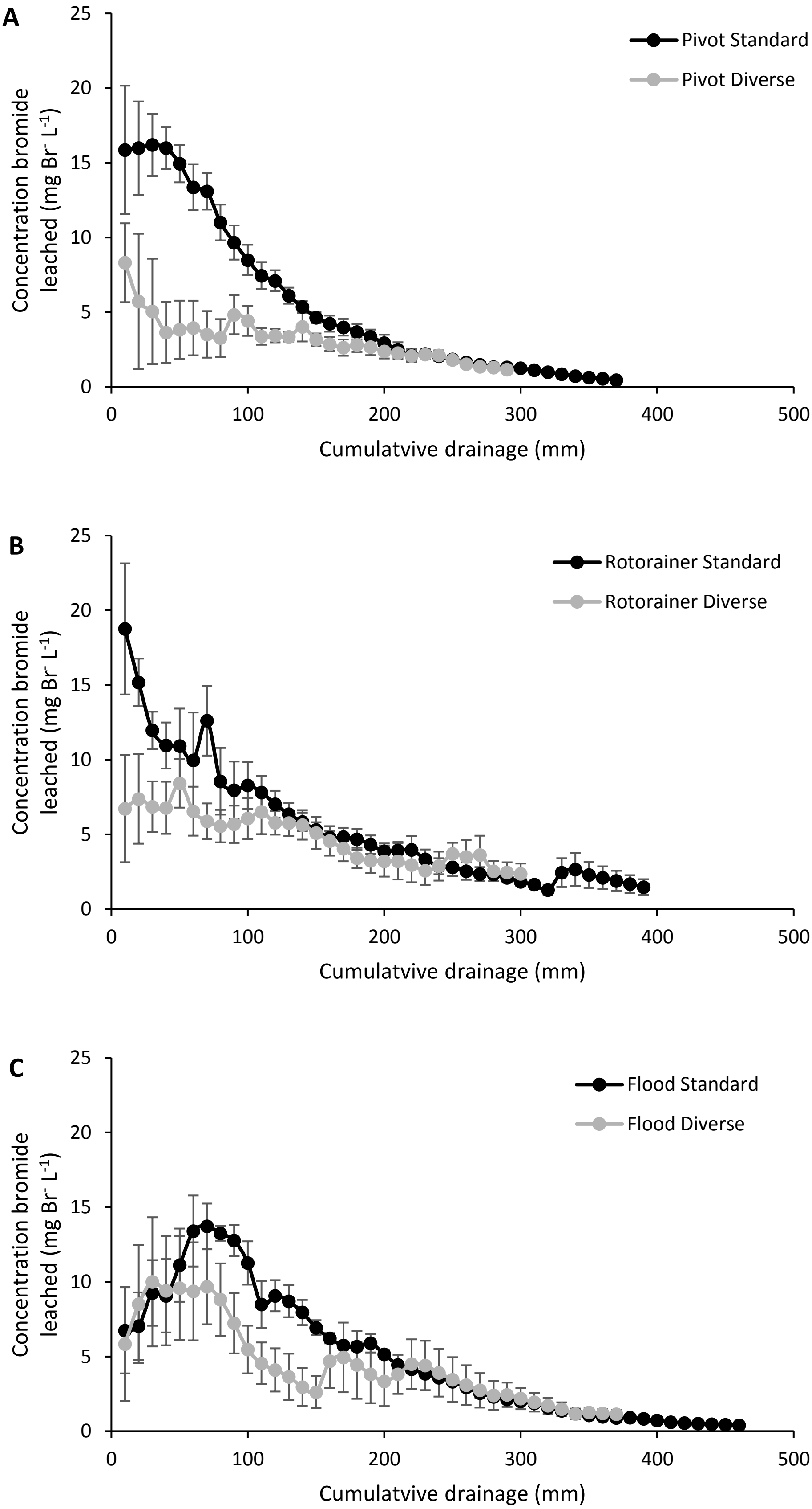


Figure 6.10. Concentration of Br^- (mg L^{-1}) in drainage water following a December urine application as affected by pivot irrigation (A), rotorainer irrigation (B) and flood irrigation (C). Error bars are ± SEM.

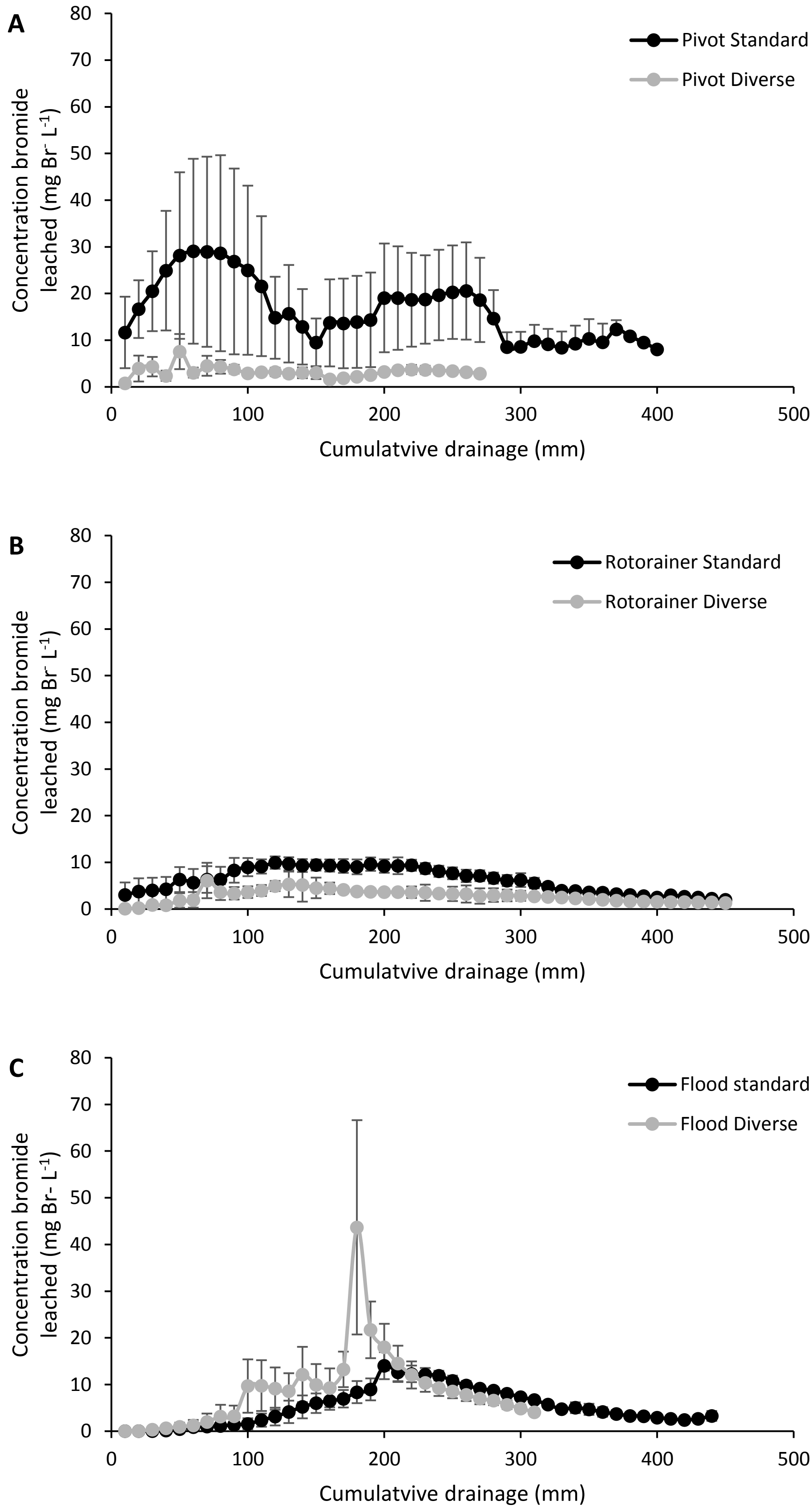


Figure 6.11. Concentration of Br^- (mg L^{-1}) in drainage water following a February urine application as affected by pivot irrigation (A), rotorainer irrigation (B) and flood irrigation (C). Error bars are ± SEM.

6.3.8 Total herbage DM yield and N uptake

Averaged across all treatments, the total herbage DM yield (t DM ha^{-1}) was significantly ($P < 0.001$) affected by urine application month (Table 6.6). The DM yield from urine applied in December was 14% (diverse forage) and 9% (standard forage) greater than the DM yield from the February applied urine ($P < 0.05$). Averaged across all treatments, the total herbage DM was significantly ($P = 0.010$) affected by forage type (Table 6.6). The different irrigation types had no significant ($P = 0.102$) effect on DM yield (Table 6.6).

Averaged across all treatments, the total N uptake (kg N ha^{-1}) was significantly ($P < 0.001$) affected by urine application month (Table 6.6). The N uptake from urine applied in December was 15% (diverse forage) and 16% (standard forage) greater than the herbage N uptake from the February applied urine ($P < 0.05$). Averaged across all treatments, the total herbage N uptake was significantly ($P = 0.021$) affected by forage type (Table 6.6). The different irrigation types had no significant ($P = 0.061$) effect on herbage N uptake.

December urine application

Following the December urine application, the total herbage DM yield and N uptake ranged from 13.3–14.9 t DM ha^{-1} and 408–466 kg N ha^{-1} (standard forage) compared to 12.2–14.2 t DM ha^{-1} and 355–441 kg N ha^{-1} (diverse forage) (Figure 6.12A and Figure 6.13A). There was no significant difference in herbage DM yield and N uptake between irrigation treatments.

February urine application

Following the February urine application, the total herbage DM yield and N uptake ranged from 12.4–13.6 t DM ha^{-1} and 358–405 kg N ha^{-1} (standard forage) compared to 11.1–12.8 t DM ha^{-1} and 308–397 kg N ha^{-1} (diverse forage) (Figure 6.12B and Figure 6.13B). Under the diverse forage, N uptake was greater under the pivot irrigation treatment compared with the flood irrigation treatment ($P < 0.05$). There was no significant difference in herbage DM yield between irrigation treatments.

Table 6.6. Total herbage DM yield (kg DM ha^{-1}) and herbage N uptake (kg N ha^{-1}) from lysimeters as affected by irrigation type, forage type and urine application month.

Irrigation	Forage	Urine app. month	Log$_{10}$ means DM yield (kg DM ha^{-1})	Log$_{10}$ means N uptake (kg N ha^{-1})
Pivot	Diverse	December	4.147	2.635
	Standard	December	4.176	2.667
	Diverse	February	4.108	2.595
	Standard	February	4.117	2.590
Rotorainer	Diverse	December	4.150	2.636
	Standard	December	4.172	2.664
	Diverse	February	4.050	2.544
	Standard	February	4.131	2.606
Flood	Diverse	December	4.086	2.549
	Standard	December	4.122	2.610
	Diverse	February	4.042	2.493
	Standard	February	4.087	2.547
LSD (5%) within irrigation regimes			0.068	0.080
LSD (5%) for all other comparisons			0.074	0.087
Irrigation			NS	NS
Forage			**	*
Urine app. month			***	***
Irrigation × forage			NS	NS
Irrigation × urine app. month			NS	NS
Forage × urine app. month			NS	NS
Irrigation × forage × urine app. month			NS	NS

NS = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

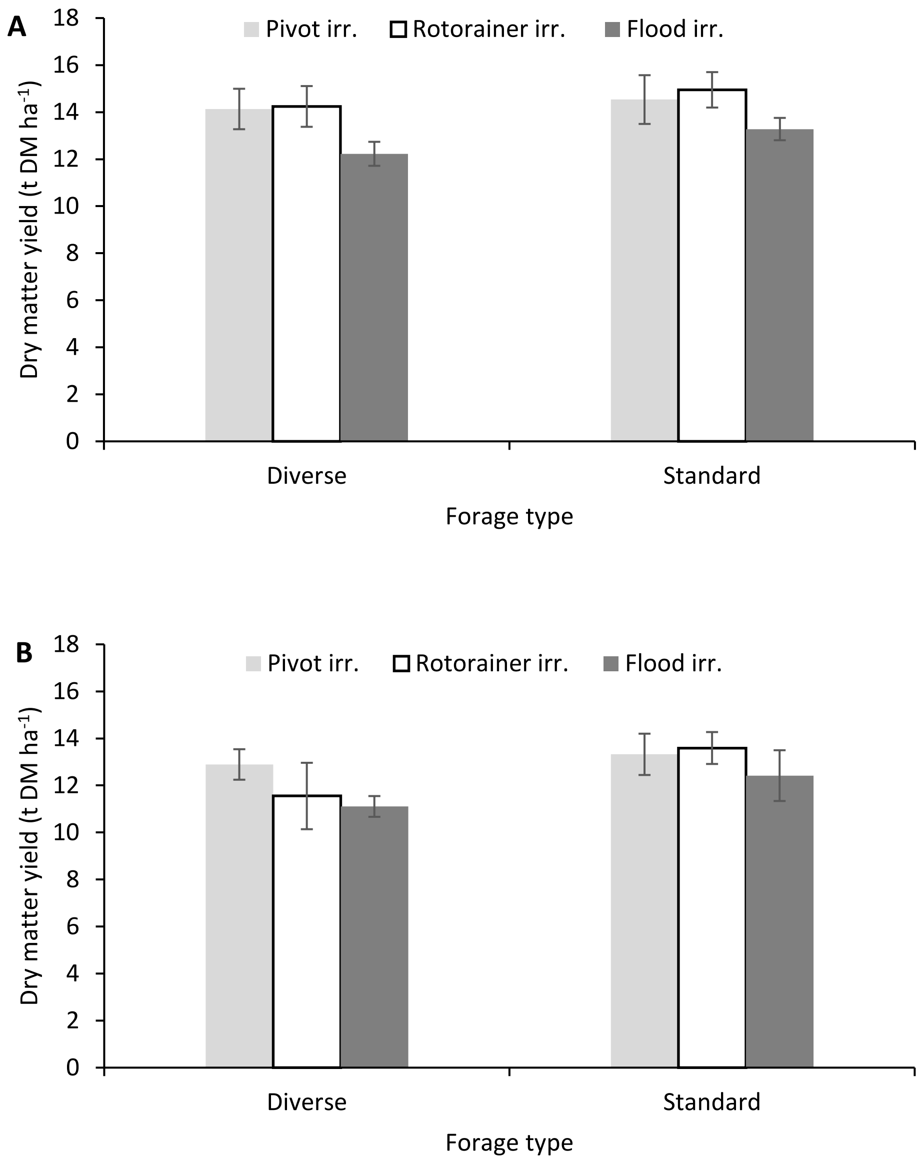


Figure 6.12. Total DM yield (t DM ha^{-1}) from lysimeters as affected by irrigation (pivot vs. rotorainer vs. flood), forage type (standard vs. diverse) and urine application date (December (A) and February (B)). Error bars are ± SEM.

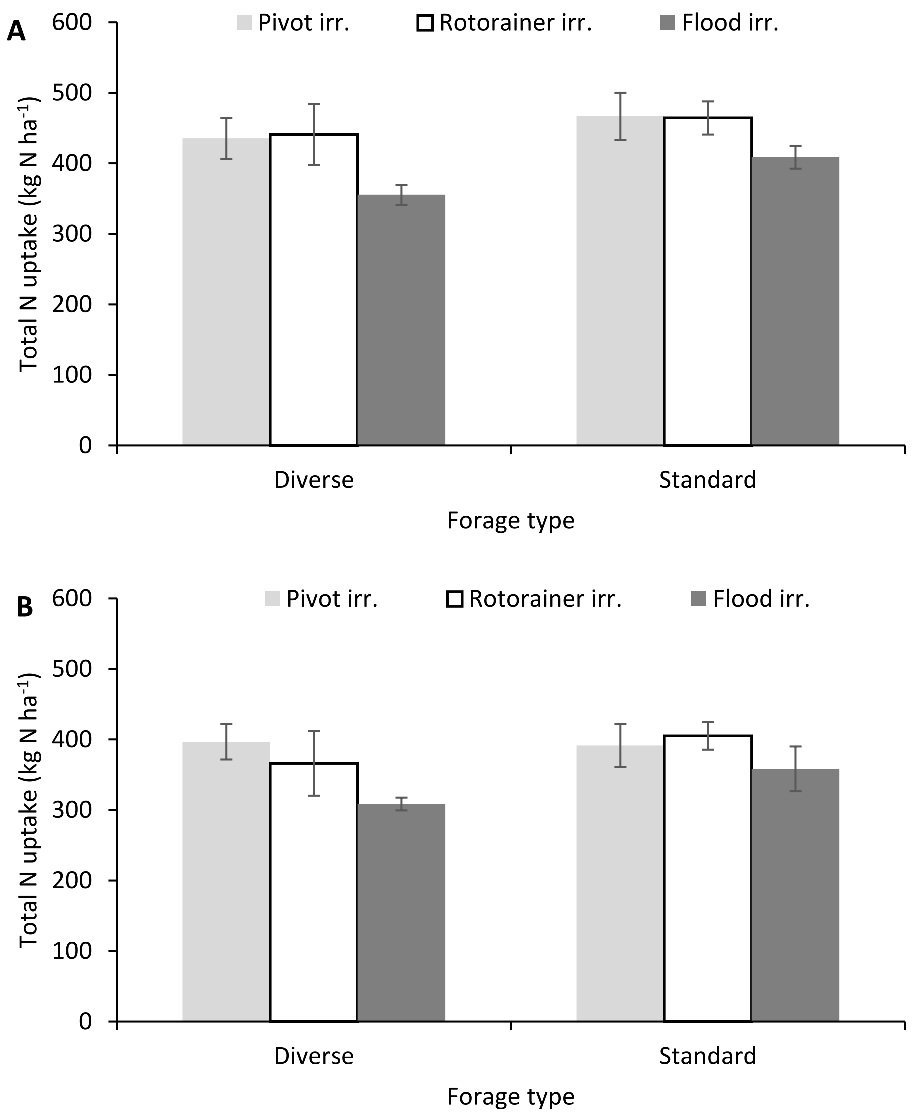


Figure 6.13. Total N uptake (kg N ha^{-1}) from lysimeters as affected by irrigation (pivot vs. rotorainer vs. flood), forage type (standard vs. diverse) and urine application date (December (A) and February (B)). Sample size is 3016 cm^3 soil. Error bars are ± SEM.

6.3.9 Monthly herbage N uptake

Following urine application in December herbage N uptake was greatest in January for all irrigation treatments (Figure 6.14). Following urine application in February, herbage N uptake was greatest in March and April with lower N uptake in the winter months (Figure 6.15). There were no differences in monthly herbage N uptake between the standard and diverse forage types (Figure 6.14 & Figure 6.15).

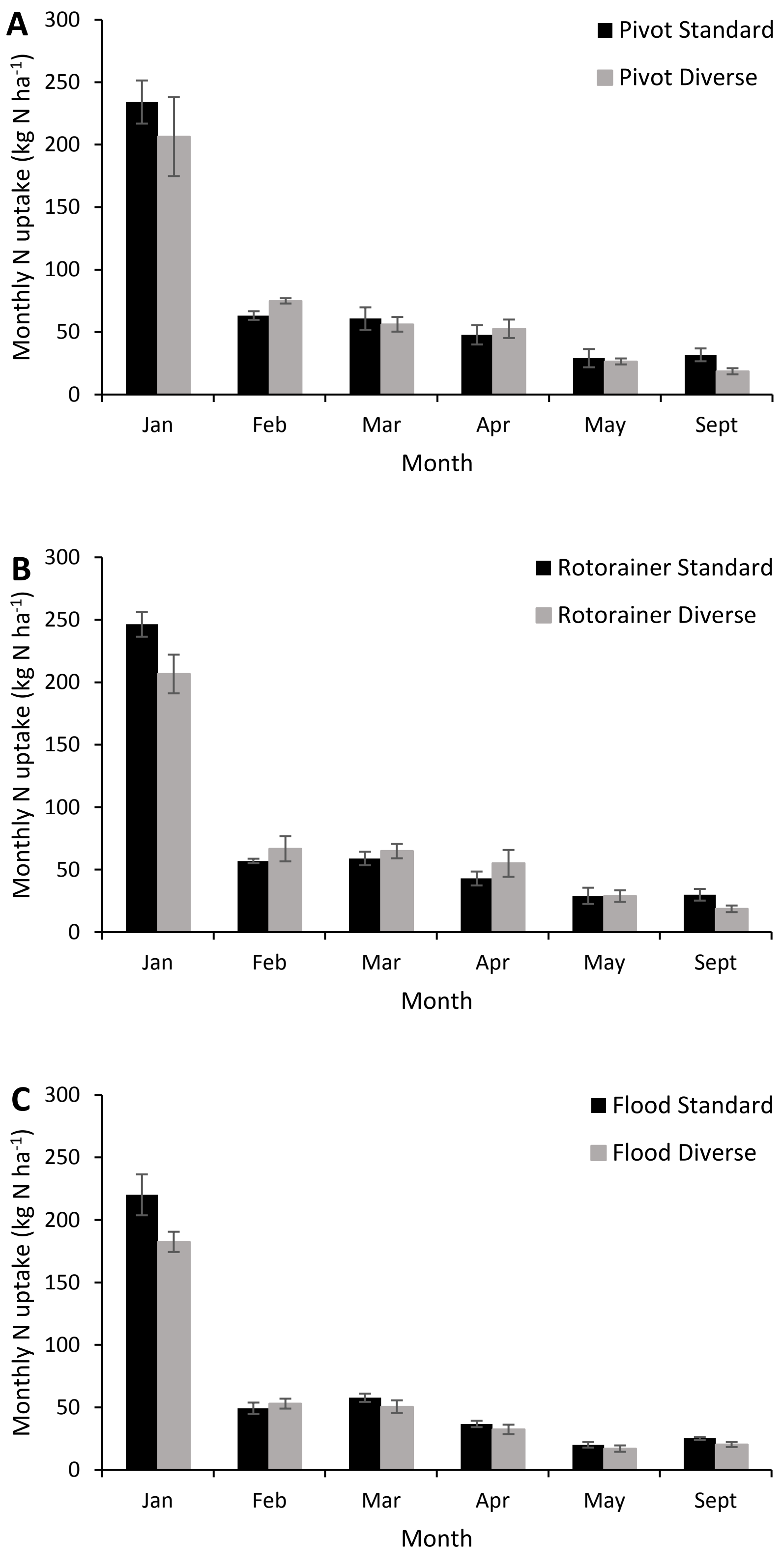


Figure 6.14. Monthly N uptake (kg N ha^{-1}) following urine application in December as affected by pivot irrigation (A), rotorainer irrigation (B) and flood irrigation(C). Error bars are ± SEM.

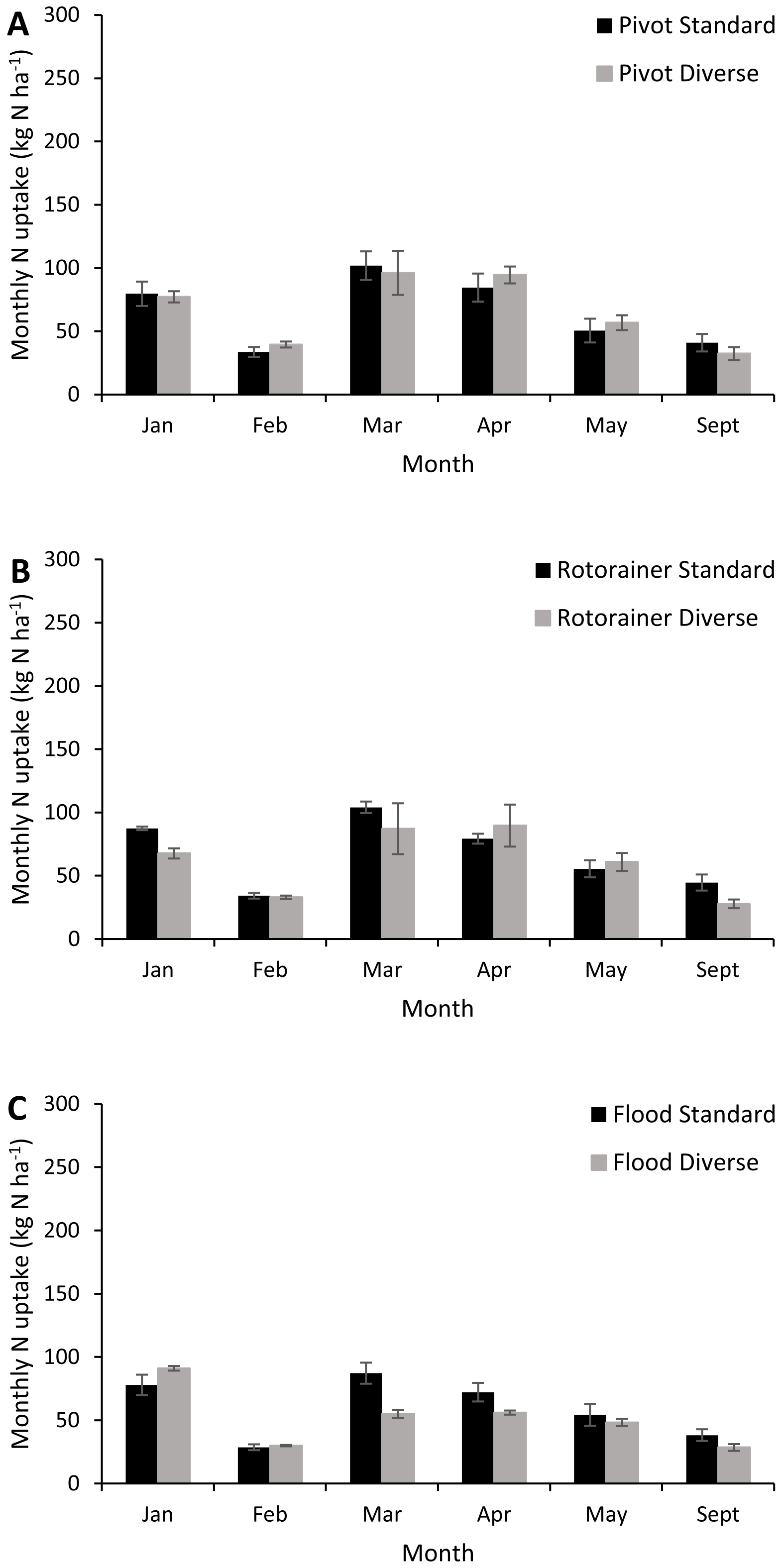


Figure 6.15. Monthly N uptake (kg N ha^{-1}) following urine application in February as affected by pivot irrigation (A), rotorainer irrigation (B) and flood irrigation(C). Error bars are ± SEM.

6.3.10 Root distribution

Root volume

There was no significant difference in root volume between treatments (Table 6.7). The total volume of recovered roots ranged from 7.4-8.6 cm^3 (diverse forage) and 7.5-11.8 cm^3 (standard forage) for a 3016 cm^3 sample of soil (Figure 6.16).

Table 6.7. Total root volume (cm^3), root length (m) and surface area (cm^2) from lysimeters at the end of the experimental period. Sample size is 3016 cm^3 soil.

Irrigation	Forage	Urine app. month	Total volume (cm^3)	Total length (m)	Total surface area (cm^2)
Pivot	Diverse	December	8.17	95.6	973
	Standard	December	8.88	152.7	1283
	Diverse	February	7.60	92.5	930
	Standard	February	9.73	165.5	1411
Rotorainer	Diverse	December	8.28	59.7	755
	Standard	December	7.56	144.1	1163
	Diverse	February	7.92	98.2	981
	Standard	February	9.20	138.5	1250
Flood	Diverse	December	7.47	84.8	878
	Standard	December	9.67	152.7	1352
	Diverse	February	8.34	80.8	899
	Standard	February	11.83	185.6	1652
LSD (5%) within irrigation regimes			4.03	48.3	463.4
LSD (5%) for all other comparisons			6.66	45.5	423.1
<u>Significance of main effect</u>					
Irrigation			NS	NS	NS
Forage			NS	***	***
Urine app. month			NS	NS	NS
Irrigation × forage			NS	NS	NS
Irrigation × urine app. month			NS	NS	NS
Forage × urine app. month			NS	NS	NS
Irrigation × forage × urine app. month			NS	NS	NS

NS = not significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

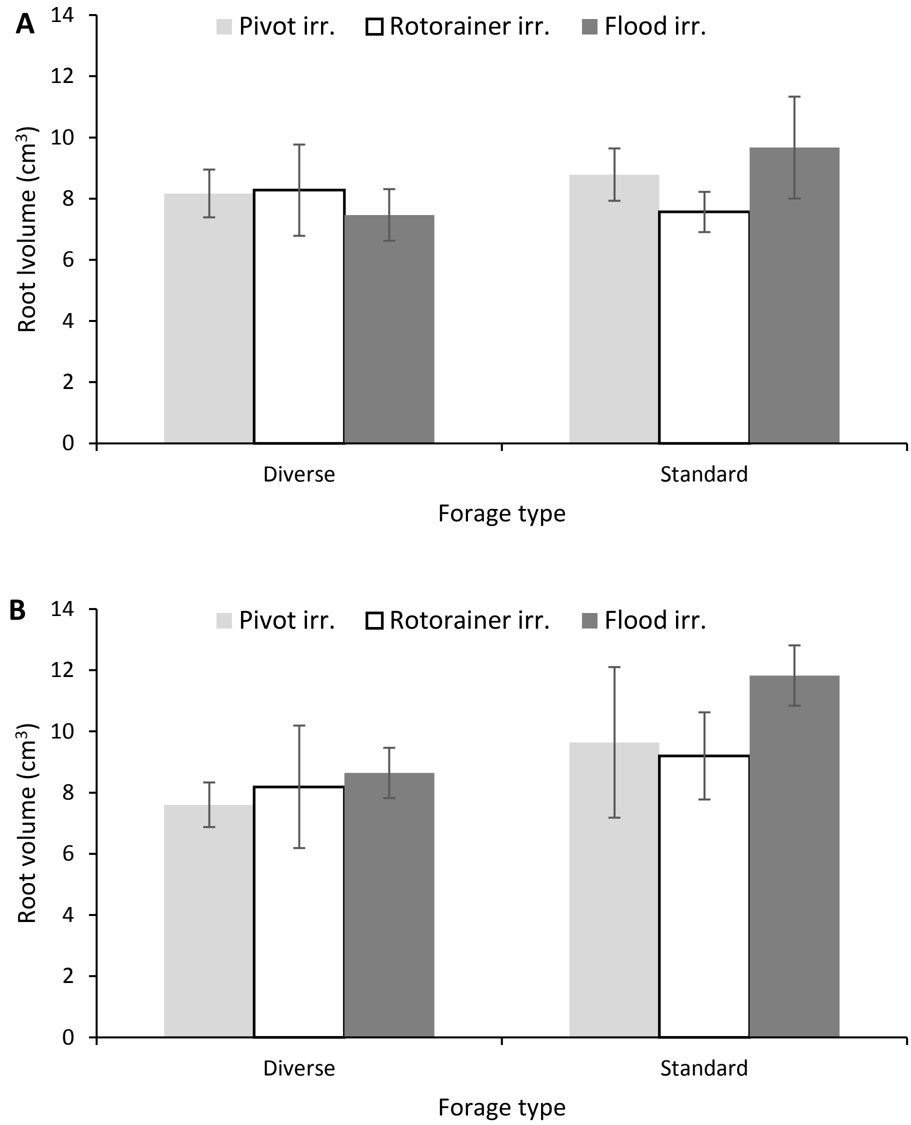


Figure 6.16. Total root volume (cm^3) from lysimeters as affected by irrigation (pivot vs. rotorainer vs. flood), forage type (standard vs. diverse) and urine application date (December (A) and February (B)). Sample size is 3016 cm^3 soil. Error bars are ± SEM.

Root surface area

Averaged across all treatments, total root surface area (cm^2) was affected by forage type ($P < 0.001$) (Table 6.7). The total root surface area ranged from 755–988 cm^2 (diverse forage) and 1163–1652 cm^2 (standard forage) for a 3016 cm^3 sample of soil (Figure 6.17). Under the flood irrigation treatment, total root surface area was significantly ($P < 0.05$) less in the diverse forage compared to the standard forage. Under the pivot irrigation treatment, root surface area was significantly ($P < 0.05$) less in the diverse forage compared to the standard forage when urine was applied in December ($P < 0.05$) (Figure 6.17). Irrigation type had no effect on total root surface area ($P = 0.127$).

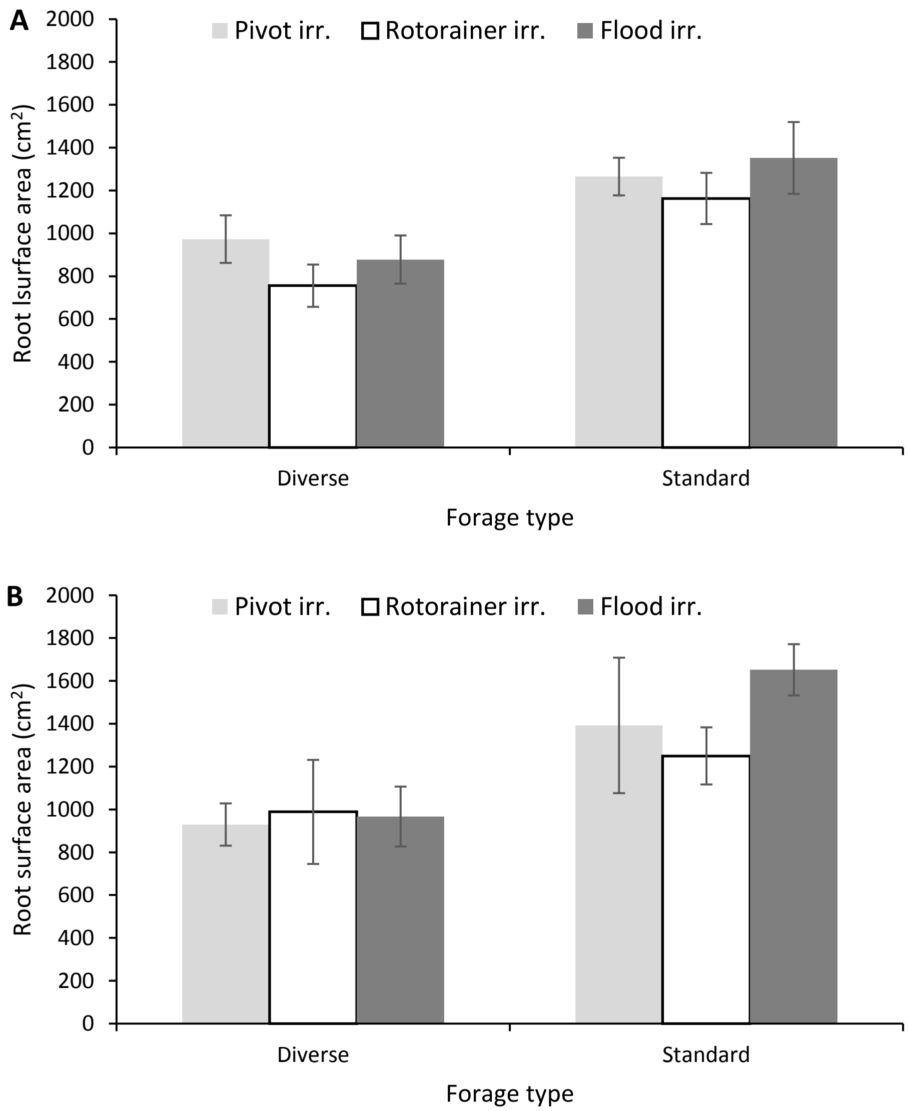


Figure 6.17. Total root surface area (cm^2) from lysimeters as affected by irrigation (pivot vs. rotorainer vs. flood), forage type (standard vs. diverse) and urine application date (December (A) and February (B)). Sample size is 3016 cm^3 soil. Error bars are ± SEM.

Root length

Averaged across all treatments, total root length (m) was affected by forage type (P < 0.001) (Table 6.7). The total root length ranged from 60–96 m (diverse forage) and 138–185 m (standard forage) for a 3016 cm^3 sample of soil (Figure 6.18). Under all irrigation treatments, the total root length was significantly (P < 0.05) lower for the diverse forage compared to the standard forage when urine was applied in December. Similarly, under pivot and flood irrigation treatments, the total root length was significantly (P < 0.05) lower for the diverse forage compared to the standard forage when urine was applied in February (Figure 6.18). Irrigation type had no effect on total root surface area (P = 0.207).

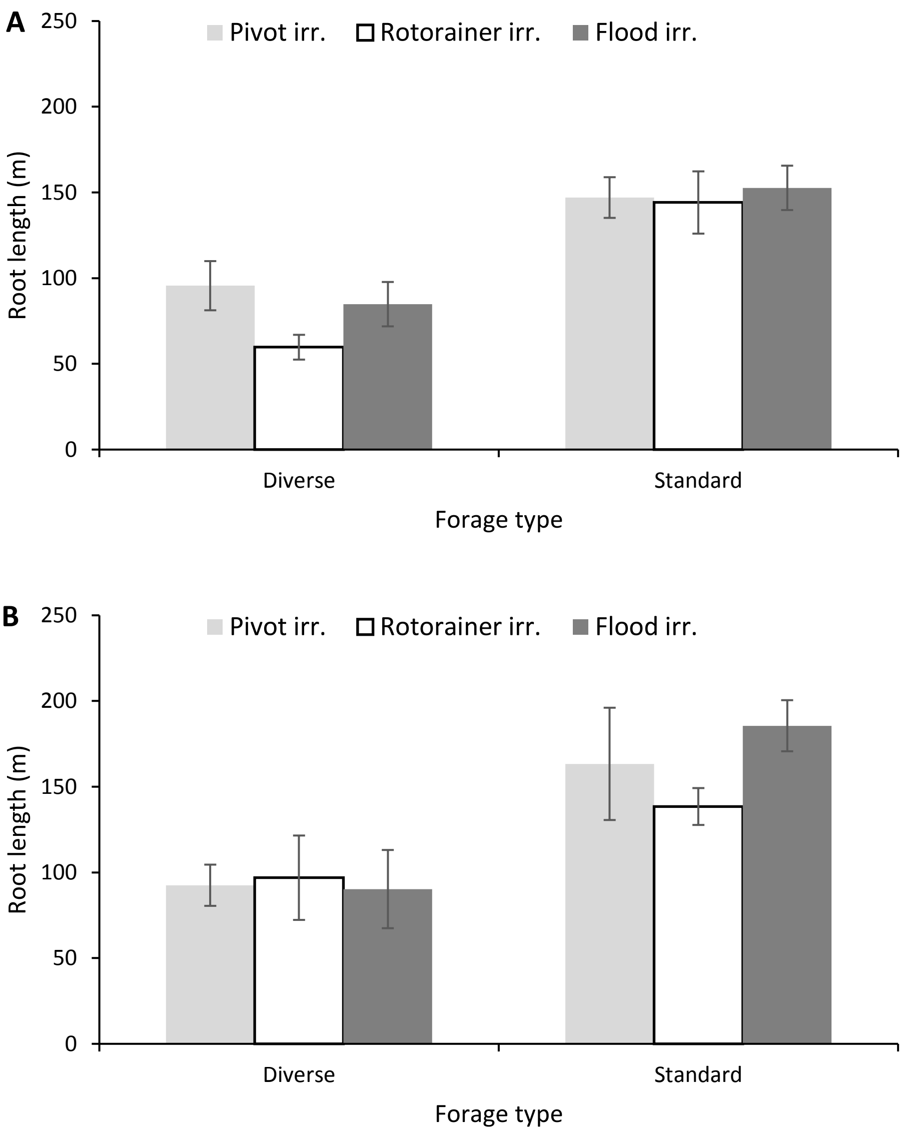


Figure 6.18. Total root length (m) from lysimeters as affected by irrigation (pivot vs. rotorainer vs. flood), forage type (standard vs. diverse) and urine application date (December (A) and February (B)). Sample size is 3016 cm^3 soil. Error bars are ± SEM.

Root length density

Averaged across all treatments, root length density (cm cm^{-3}) was affected by forage type at all depths ($P < 0.001$) (Table 6.8). At 0–100 mm, total root length density ranged from 8–16 cm cm^{-3} (diverse forage) and 21–29 cm cm^{-3} (standard forage) (Figure 6.19). The total root length density between 100 mm and 600 mm was less than 3.5 cm cm^{-3} for all treatments. At 0–100 mm, under flood irrigation, the root length density of the standard forage was 110% (December application) and 67% (February application) greater than the root length density of the diverse forage ($P < 0.05$). A similar trend was observed under rotorainer irrigation when urine was applied in December ($P < 0.05$), and pivot irrigation when urine was applied in February ($P < 0.05$). Irrigation type had no effect on root length density.

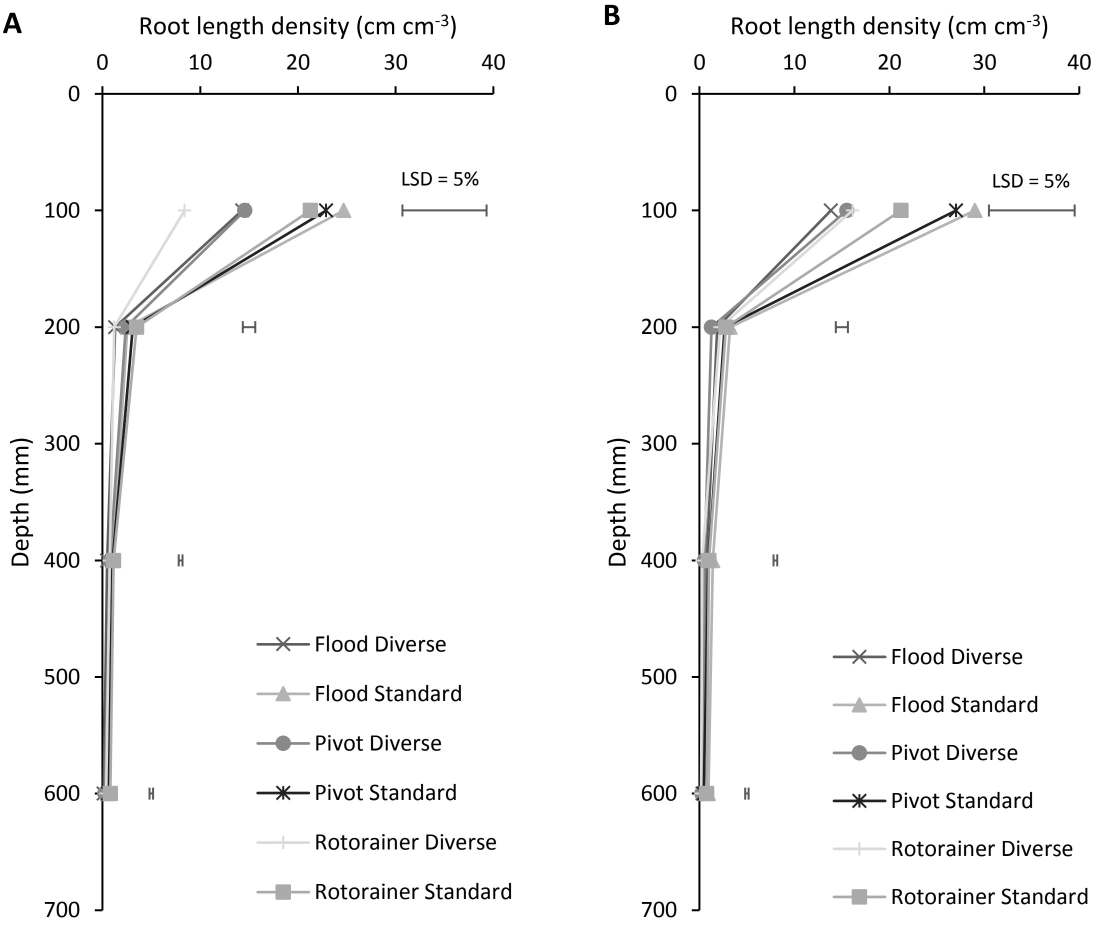


Figure 6.19. Total root length density (cm cm^{-3}) from lysimeters as affected by irrigation (pivot vs. rotorainer vs. flood), forage type (standard vs. diverse) and urine application date (December (A) and February (B)). Sample size is 3016 cm^3 soil. LSD ($P < 0.05$) calculated from treatment means.

Root diameter

Averaged across all treatments, root diameter (mm) was affected by forage type at all depths (P < 0.001). At 0–100 mm there was a significant interaction between forage type and urine application date, irrigation type and urine application date, and irrigation, forage type and urine application date (Table 6.8). At 0–100 mm, root diameter ranged from 0.31–0.47 mm (diverse forage) and 0.23–0.29 mm (standard forage) (Figure 6.20). At 0–100 mm, the root diameter of the diverse forage was significantly (P < 0.05) greater than the root diameter of the standard forage for all irrigation types (December urine application). At 0–100 mm, the root diameter of the diverse forage was significantly (P < 0.05) greater than the root diameter of the standard forage under flood irrigation (February urine application) (Figure 6.20). Irrigation type had no effect on root diameter.

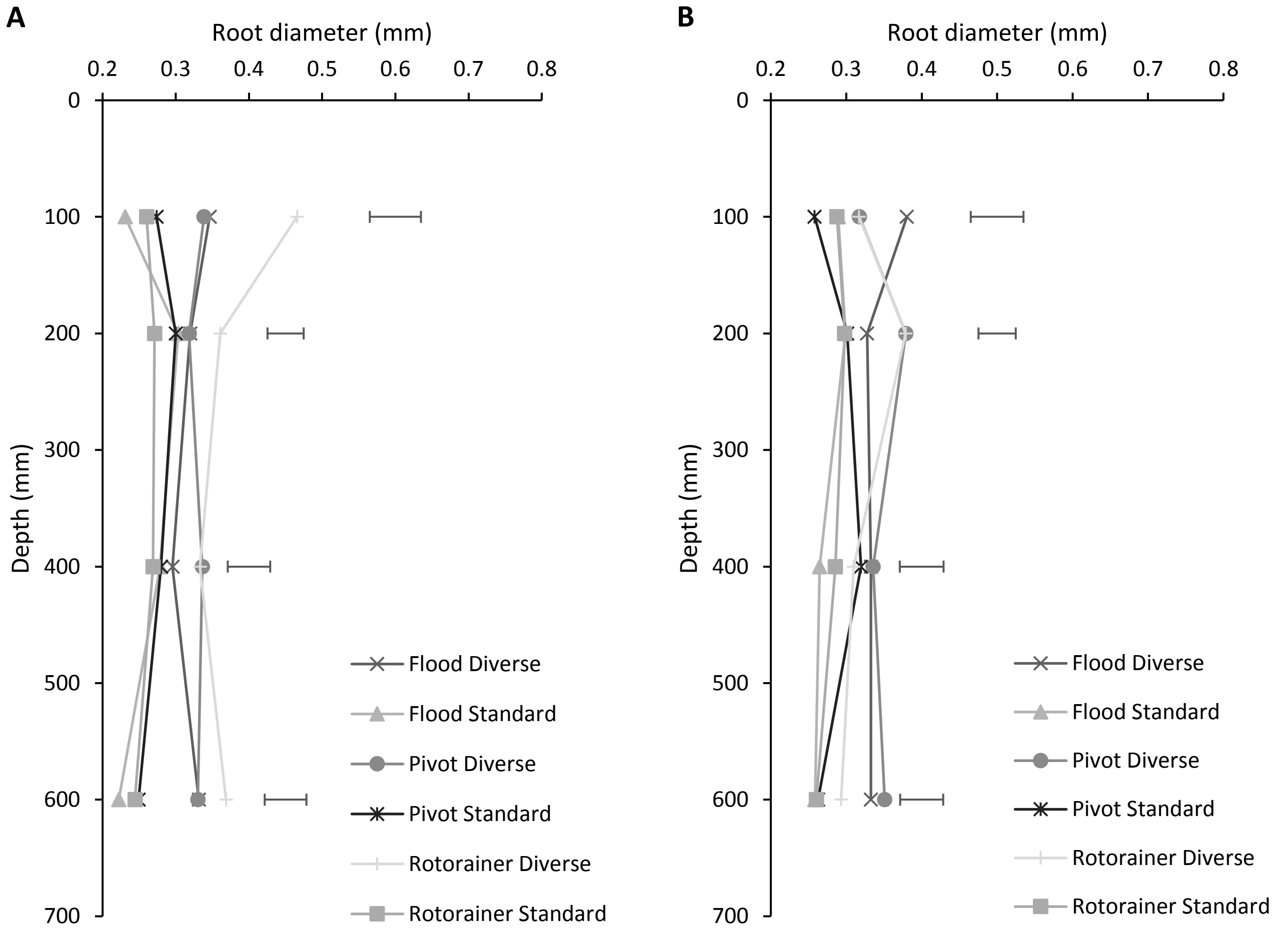


Figure 6.20. Average root diameter (mm) from lysimeters as affected by irrigation (pivot vs. rotorainer vs. flood), forage type (standard vs. diverse) and urine application date (December (A) and February (B)). Sample size is 3016 cm^3 soil. LSD (P <0.05) calculated from treatment means.

Table 6.8. Root length density (cm cm^{-3}) and root diameter (mm) at depths of 0-100 mm, 100-200 mm, 200-400 mm and 400-600 mm from lysimeters at the end of the experimental period. Sample size is 3016 cm^3 soil.

Irrigation	Forage	Urine rate	Root length density (cm cm^{-3})				Root diameter (mm)			
			0-100	100-200	200-400	400-600	0-100	100-200	200-400	400-600
Pivot	Diverse	December	14.57	2.37	0.77	0.26	0.339	0.318	0.336	0.330
	Standard	December	23.37	3.01	0.99	0.66	0.271	0.299	0.284	0.259
	Diverse	February	15.53	1.27	0.53	0.28	0.318	0.379	0.336	0.351
	Standard	February	27.49	2.56	0.91	0.53	0.255	0.300	0.324	0.272
Rotorainer	Diverse	December	8.40	1.21	0.75	0.39	0.466	0.361	0.333	0.368
	Standard	December	21.27	3.50	1.15	0.81	0.260	0.271	0.269	0.244
	Diverse	February	16.50	2.10	0.29	0.18	0.307	0.379	0.303	0.290
	Standard	February	21.22	2.78	1.00	0.77	0.288	0.298	0.286	0.260
Flood	Diverse	December	14.29	1.31	0.50	0.14	0.346	0.319	0.295	0.331
	Standard	December	24.68	2.56	1.07	0.51	0.231	0.303	0.278	0.222
	Diverse	February	12.03	1.93	0.76	0.30	0.400	0.337	0.338	0.333
	Standard	February	29.01	3.22	1.39	0.95	0.289	0.299	0.265	0.259
LSD (5%) within irrigation regimes			9.03	1.32	0.41	0.32	0.066	0.043	0.058	0.055
LSD (5%) for all other comparisons			8.61	1.28	0.39	0.36	0.070	0.040	0.058	0.057
<u>Significance of main effect</u>										
Irrigation			NS	NS	NS	NS	NS	NS	NS	NS
Forage			***	***	***	***	***	***	***	***
Urine app. month			NS	NS-	NS	NS	NS	*	NS	NS
<u>Significance of interaction</u>										
Irrigation × forage			NS	NS	NS	NS	NS	*	NS	NS
Irrigation × urine app. month			NS	NS	NS	*	**	NS	NS	NS
Forage × urine app. month			NS	NS	NS	NS	*	NS	NS	NS
Irrigation × forage × urine app. month			NS	NS	NS	NS	*	NS	NS	NS

NS = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

6.4 Discussion

6.4.1 Nitrate leaching losses

The results from this experiment show that NO_3^- leaching losses from the diverse forage were 82% less than the standard forage when urine was applied in December (early summer) and 74% less when urine was applied in February (late summer). Interestingly, the type of irrigation system used had no significant effect on NO_3^- leaching losses from either the standard or diverse forage types grown on this Paparua fine sandy loam. Furthermore, a greater amount of NO_3^- leaching occurred under the diverse forage when urine was applied in February (late summer) compared with December (early summer). Although there was a trend for a greater amount of NO_3^- leaching under the standard forage when urine was applied in February, this difference was not significant.

The effects of forage type, irrigation type and urine application date on plant N uptake and NO_3^- leaching losses are discussed below.

6.4.2 Effect of forage type

The results from Chapter Five show that AOB abundance was lower under the diverse forage containing plantain compared with the standard perennial ryegrass and white clover forage. Consequently, the soil NH_4^+–N concentrations remained greater under the diverse forage whilst the soil NO_3^-–N concentration was lower. The lower NO_3^-–N concentrations under the diverse forage were attributed to the release of BNI compounds into the soil by the plantain. Other studies have also observed BNI in the presence of plantain, or plantain derived compounds such as aucubin, however, these studies were all associated with low N environments (Rauber *et al.*, 2008; Dietz *et al.*, 2013; Massaccesi *et al.*, 2015). Nitrification determines the amount of NO_3^- present in the soil and therefore how N is utilised or dispersed into the environment. If there is less NO_3^-–N present in soil solution the risk of NO_3^- leaching can be reduced (Cameron *et al.*, 2013). The lower NO_3^- leaching losses observed under the diverse forage in this experiment may therefore be attributed to an initial reduction in soil NO_3^-–N concentration resulting from the release of BNI substances into the soil by plantain. This has been demonstrated in studies using commercial nitrification inhibitors, such as dicyandiamide (DCD), where the application of a nitrification inhibitor has resulted in significant reductions in NO_3^- leaching losses from cow urine (Di & Cameron, 2002b; Di & Cameron, 2007; Di *et al.*, 2010; Di & Cameron, 2012). For example, Di and Cameron (2002b) found that DCD applied in late spring reduced NO_3^- leaching losses from cow urine patches by 42%. The observed reduction in NO_3^- losses from the DCD treated forage was less than the reduction in NO_3^- leaching losses observed in this experiment. This could be the result of DCD having a shorter half-life when soil temperature increases during the summer months (Di & Cameron, 2002b).

Interestingly, the nitrification inhibition and subsequently higher NH_4^+–N concentrations that were observed under the diverse forage did not result in greater N uptake by the diverse forage compared with the standard perennial ryegrass and white clover forage. Ammonium has a positive charge, thus it is readily adsorbed onto soil exchange surfaces. This can result in a greater opportunity for NH_4^+ to be immobilised into soil organic matter or fixed into 2:1 type minerals, rather than being leached (McLaren & Cameron, 1996). Greater N immobilisation has been observed in studies using commercial nitrification inhibitors, such as DCD (Juma & Paul, 1983; Vilsmeier, 1991; Soliman & Abdel Monem, 1995; Xu *et al.*, 2000). Vilsmeier (1991) found that the application of DCD resulted in increased immobilisation, with 50% of the applied N being recovered in the soil compared with only 39% in the non-DCD treated soils. Similarly, Xu *et al.* (2000) found that more fertiliser derived N was recovered in the soil when a nitrification inhibitor (DCD) was used together with a urease inhibitor (N-[n-butyl] thiophosphoric triamide) (34.3–50.6%, in contrast to 9.9% in the absence of inhibitors). It is also possible that in this experiment N was immobilised in the roots of the diverse forage. Following urine application (with or without dairy farm effluent), Di *et al.* (2002) found that 19–20% of the N applied was retained in the pasture roots one year after application. The immobilisation of N may therefore help to account for the lower NO_3^- leaching losses that were observed in this experiment under the diverse forage. However, it is important to note that N which has been immobilised could be mineralised and made available in the longer term.

The total volume of drainage under the diverse forage was markedly less than the volume of drainage under the standard forage. The lower drainage volumes under the diverse forage may be the result of greater water uptake by the plantain. For example, under optimum irrigation, Neal *et al.* (2011) found that plantain had a higher total water use (1,173 vs. 956 mm) and lower water use efficiency (13.6 vs. 16.3 kg ha^{-1} mm^{-1}) compared with perennial ryegrass when grown in a monoculture. In addition, under deficit irrigation, Neal *et al.* (2012) measured greater soil moisture deficits under plantain with increasing soil depth when compared to perennial ryegrass and suggested that species such as plantain could reduce the risk of deep drainage taking place. Carey *et al.* (2016) has also reported an indirect benefit of reduced drainage on NO_3^- leaching losses from oat catch crops. In this study Carey *et al.* (2016), found that drainage volumes were lowest for the earliest sown oats compared with the corresponding fallow treatment and/or later-sown treatments. It was suggested that while N uptake from the soil was the primary benefit of sowing a catch crop, there was an indirect benefit of reduced drainage because evaporative water loss from the growing crop exceeded surface soil evaporation alone. The lower drainage volumes in combination with the BNI effect are therefore likely to account for the lower NO_3^- leaching losses observed under the diverse forage containing plantain.

On a similar soil type, Silva *et al.* (2000) measured that one pore volume of drainage occurred at approximately 300 mm of drainage. The drainage volumes collected in this experiment were therefore close to or exceeded one pore volume of drainage for all treatments, thus if NO_3^- was present in the soil it is probable that it would have been collected in the leachate. This suggests that other mechanisms, such as possible BNI effects, had a more substantial influence on NO_3^- leaching losses than drainage volumes.

The recovery of bromide in the leachate ranged from 16–54% of the Br^- applied and was affected by forage type with lower Br^- recovery occurring under the diverse forage. While Br^- recovery in herbage was not measured in this experiment it is probable that plant uptake would have accounted for a proportion of the Br^- that was not recovered in the leachate. For example, when Br^- was applied as a tracer to a Russet Burbank potato (*Solanum tuberosum L.*) crop, Kung (1990) measured a 53% recovery of Br^- in the potato plant material 63 days after application. Similarly, seven weeks after planting and at harvest, 16% and 27% of the Br^- applied to a corn crop was recovered in the plant material (Kessavalou *et al.*, 1996).

Averaged across all treatments the herbage DM yield and herbage N uptake from the diverse forage was lower than the standard forage. This contrasts with the results observed in Chapter Four and those reported by Goh and Bruce (2005); Nobilly *et al.* (2013); Woodward *et al.* (2013); Malcolm *et al.* (2014) who found no difference or higher DM yields from diverse forages compared with a standard perennial ryegrass and white clover forage. In these earlier studies, the number of different plant species in the diverse forage ranged from 4 to 17 which was greater than the three plant species (perennial ryegrass, white clover and plantain) used in this experiment. These results suggest that the type of plant species used rather than the number of species present in the forage is more important in reducing NO_3^- leaching losses. This is because, while the DM yield and N uptake was greater from the standard forage, NO_3^-leaching losses were also significantly greater from the standard forage compared with the NO_3^- leaching losses from the diverse forage.

It is also possible that root architecture had an effect on herbage DM yield and herbage N uptake. The total root length, total root surface area, and root length density were greater under the standard forage compared with the diverse forage. Studies have shown that soil N interception was greater in plant species with finely divided root structures and a larger surface area (Habib & La Folie, 1991; Dunbabin *et al.*, 2003; Crush *et al.*, 2005). Dunbabin *et al.* (2003) modelled the effect of root architecture on N capture for a range of crops using ROOTMAP. The results generated, suggested that plants capable of generating a high density of roots in the top soil early in the season had the greatest potential to capture soil N. However, while this may explain the greater herbage DM yield and N uptake from the standard forage, it suggests that root architecture is <u>not</u> the main mechanism influencing NO_3^- leaching losses. This supports the hypothesis that the release of a BNI from plantain and the smaller amount of drainage had the greatest influence on reducing the NO_3^- leaching loss.

Studies have also shown that NO_3^- leaching losses can be reduced due to greater winter activity and thus N uptake by plant species such as Italian ryegrass when compared to perennial ryegrass and white clover forages (Malcolm *et al.*, 2014; Woods *et al.*, 2016). However, in this experiment there was little or no difference in monthly herbage N uptake between the standard and diverse forage types from December 2015 to September 2016. This suggests that seasonal differences in herbage N uptake did not influence the differences in NO_3^- leaching losses that were measured under the diverse and standard forage types in this experiment.

6.4.3 Effect of irrigation

The results from this experiment found that irrigation type influenced the timing of the peak NO_3^-–N concentrations in the breakthrough curves. Under the pivot and rotorainer irrigation, peak NO_3^-–N concentrations occurred at the onset of drainage and steadily declined thereafter. In contrast, under flood irrigation, there was a delay in peak NO_3^-–N concentrations, which occurred following a greater amount of drainage. The difference in the timing of the NO_3^-–N breakthrough curves was probably due to differences in solute transport mechanisms between the three irrigation types.

Under the frequent but lower rates of irrigation (pivot and rotorainer), it is possible that NO_3^- was displaced gradually down the soil profile, via slow matrix flow, with little or no drainage occurring during the irrigation period (December to April). As a result, peak NO_3^-–N concentrations were elevated with the onset of drainage during autumn and winter. This has been observed in a field study using dye to characterise preferential flow of water in an undisturbed soil, where sprinkler irrigation resulted in a uniform complete leaching of dye away from the soil surface (> 20 cm) (Ghodrati & Jury, 1990). Ghodrati and Jury (1990) further observed that under flood irrigation, for the most part, the dye remained in the top 10 cm of the soil surface. It was suggested that the majority of the water added drained rapidly through a relatively small fraction of the soil via preferential flow channels therefore leaving the majority of the dye at the soil surface. Similar results were observed by Watson and Luxmoore (1986), who estimated that under ponded flow, 96% of the soil water flux was transported through only 0.32% of the soil volume. It is therefore likely that inefficient matrix flow under the flood irrigation in this experiment caused the NO_3^-–N pulse to take longer to travel down the soil profile. As a result there was a delay in peak NO_3^-–N leaching compared with the pivot and rotorainer irrigation. Furthermore, preferential flow has been well documented under flood irrigation (Bowman & Rice, 1986; Fraser *et al.*, 1994; Mohanty *et al.*, 1998; Abbasi *et al.*, 2003). This experiment showed that preferential flow occurred under flood irrigation during the summer period. The transport of NO_3^-–N in preferential flow is therefore the probable reason for the initial smaller peaks observed in the NO_3^-–N breakthrough curve under flood irrigation.

Interestingly, the flood irrigation did not result in significantly greater total NO_3^- leaching than the pivot and rotorainer irrigation. This was unexpected and differed to the findings of Moore (2002) and Daudén *et al.* (2004) who observed greater NO_3^-–N leaching losses under flood irrigation systems. However, in these studies the total amount of water applied under flood irrigation was often double the amount applied under the spray irrigation treatments. In contrast, there was only a small difference in the total irrigation water applied between the pivot (422 mm), rotorainer (468 mm) and flood (555 mm) irrigation in this experiment. Irrigation type also had no significant effect on herbage DM yield and N uptake for both forage types. Similar results were reported by Uçan *et al.* (2007) who found that irrigation frequency (7, 14 or 21 days) had no significant effect on sesame (*Sesamum indicum* L.) seed yield. These results suggest that if an optimum amount of water is applied over the season, which matches plant requirements, the irrigation system used should have no significant effect on NO_3^- leaching losses, herbage DM yield and herbage N uptake for both standard and diverse forages. However, it is important to note that this experiment was carried out on a Paparua fine sandy loam soil. It is possible that results would vary depending on soil type.

6.4.4 Effect of urine application date

The December (early summer) urine application resulted in a greater herbage DM yield and herbage N uptake than the February (late summer) urine application date, for both forage types. The significantly lower NO_3^- leaching losses from the December urine application (diverse forage) were therefore mainly a result of greater herbage N uptake and consequently lower soil mineral N concentrations remaining in the soil. Although there was a trend for lower NO_3^- leaching losses from the December urine application (standard forage), however, this was not significant. The greater herbage DM yield and herbage N uptake under the December urine application resulted from a longer period time spent under favourable growing conditions i.e. warm temperatures and longer daylight hours (Brougham, 1959). An increase in herbage DM yield and N uptake under the December application also shows the response of both forages to N availability in the soil. From urine inputs of 0 up to 1000 kg N ha^{-1}, Di and Cameron (2007) reported a significant linear relationship between the urine N rate applied and the annual pasture DM yield.

The amount of NO_3^- leached from the December urine application was similar to those reported by Decau *et al.* (2003), Stout (2003) and Decau *et al.* (2004) for summer deposited urine under irrigation. However, the NO_3^- leaching losses from both the December and February urine applications were low compared to those typically reported from autumn deposited urine (Selbie *et al.*, 2015). Irrigation, when applied at an optimum rate, may therefore be useful in capturing N during the late summer period thus reducing the potential for NO_3^- leaching over the winter period.

6.5 Conclusions

The main conclusions drawn from this experiment are:

- Nitrate leaching losses were lower under the diverse forage containing plantain compared with the standard forage.

- Lower NO_3^- leaching losses under the diverse forage were attributed to a combination of the release of biological nitrification inhibiting compounds into the soil by plantain and greater water use by plantain resulting in less drainage.

- Irrigation type had no effect on herbage DM yield, herbage N uptake and NO_3^- leaching losses under diverse and standard forage types.

- Nitrate leaching losses were lower from urine applied in early summer compared with urine applied in late summer.

- These results demonstrate the potential for diverse forages that contain plantain to mitigate NO_3^- leaching losses from cow urine patches. Furthermore, these results demonstrate that diverse forages containing plantain are able to perform well under a range of irrigation types used in New Zealand.

Chapter 7

General discussion and conclusions

7.1 General discussion

This chapter will discuss the main findings of the hypothesis testing.

7.1.1 Effect of irrigation management

Hypothesis #1: That 'optimum' irrigation will increase herbage N uptake and therefore reduce nitrate (NO_3^-) leaching losses from spring deposited urine compared with 'deficit' irrigation.

In Chapter 4, applying irrigation at an 'optimum' rate was shown to reduce NO_3^- leaching losses from spring deposited urine compared with 'deficit irrigation', thus confirming that hypothesis #1 is correct. The lower NO_3^- leaching losses under optimum irrigation were attributed to greater herbage nitrogen (N) uptake and consequently lower soil mineral N concentrations over the summer period. This was supported by the urinary ^{15}N recovery in herbage which showed that the majority of urinary applied N was recovered directly after the urine application and over the summer period. The lower NO_3^- leaching under optimum irrigation is consistent with the finding of Snow and White (2013) who modelled lower NO_3^- leaching from cow urine patches under irrigated forages when compared with dryland systems. The results from the deficit irrigation treatment also highlighted the risk of urine deposited N remaining in the soil over the summer period where it has the potential to be leached during the following winter period. These finding were in agreement with Webster and Dowdell (1984), Scholefield *et al.* (1993), Cuttle and Scholefield (1995) and Stout *et al.* (2000), who also found that NO_3^- leaching losses were greater in winters that followed a summer drought.

Hypothesis #2: That different irrigation types (pivot, rotorainer or flood) will result in different herbage N uptake, drainage and NO_3^- leaching losses.

In Chapter 6, the type of irrigation used had no significant effect on herbage N uptake, drainage or NO_3^- leaching losses from either the standard or diverse forage types, thus hypothesis #2 should be rejected. Previous research suggested that under high rates of irrigation, NO_3^- leaching losses from pasture and crops can be high, and is the result of excess water moving through the soil profile (Moore, 2002; Daudén *et al.*, 2004). However, the results from Chapter 6 suggest that provided the amount of irrigation water is not in excess of plant demand, then the type of irrigation system used (pivot, rotorainer or flood) will not increase the risk of NO_3^- leaching from summer deposited cow urine patches on a Paparua fine sandy loam soil. Similarly, irrigation type had no statistically significant effect on the herbage DM yield or herbage N uptake of either the standard or the diverse forage types. Although there is an increasing trend towards the use of pivot irrigation, rotorainer irrigation and to a lesser extent flood irrigation are still used to irrigate grazed forages in New Zealand (Irrigation New Zealand, 2017). To the author's knowledge, this is the first experiment to measure the effects of irrigation type on the herbage DM yield, herbage N uptake and NO_3^- leaching losses of a diverse forage containing plantain. These findings indicate that the productivity of diverse forages containing plantain should not be limited by the type of irrigation system used when good irrigation management is employed.

7.1.2 Effect of forage type

Hypothesis #3: That under optimum and deficit irrigation, herbage DM yield and N uptake will be greater by diverse forages due to the presence of the deep rooting species plantain and chicory.

In Chapter 4, there was no statistically significant difference in herbage DM yield and N uptake between the diverse forage and the standard forage, thus hypothesis #3 should be rejected. These results were consistent with the recent finding of Woodward *et al.* (2013) and Nobilly *et al.* (2013) who also reported no difference in herbage DM yield between a standard perennial ryegrass and white clover forage and a diverse forage which contained species similar to those used in this experiment e.g. the herbs chicory and plantain. There was also no statistically significant difference in NO_3^- leaching between the diverse and standard forages which reflects the similar herbage N uptake by the two forage types. The results from Chapter 4 suggest that root architecture and the number of plant species present in the forage may not be the key factors in reducing NO_3^- leaching. Instead individual plant characteristics may be a more important factor. This has been shown by Malcolm *et al.* (2014) and Woods *et al.* (2016) who measured lower NO_3^- leaching from Italian ryegrass and attributed this to the higher cool season activity of the Italian ryegrass. In addition, the results from Chapter 6 suggest that the release of biological nitrification inhibiting compounds by plantain is a more important factor in reducing NO_3^- leaching than root architecture.

Hypothesis #4: That NO_3^- leaching losses will be lower under diverse forages containing plantain compared with standard perennial ryegrass and white clover forages due to the release of biological nitrification inhibiting compounds by plantain.

Large reductions in NO_3^- loss (74–82%) were measured from the diverse forage containing plantain (Chapter 6), when compared to a standard perennial ryegrass and white clover forage, which confirms that hypothesis #4 is correct. This is supported by the results in Chapter 5 which showed that AOB abundance and consequently the rate of nitrification was lower in the diverse forage soil containing plantain when compared to the standard forage soil. The lower nitrification rates observed under the diverse forage are consistent with previous studies by Dietz *et al.* (2013) and Massaccesi *et al.* (2015) who also reported nitrification inhibition when plantain was present. The current experiment improves our knowledge on the biological nitrification inhibiting effects of plantain in high N environments such as cow urine patches, where the plantain released biological nitrification inhibiting compounds into the soil, and thus AOB abundance was suppressed and nitrification inhibited (Subbarao *et al.*, 2012; Dietz *et al.*, 2013). Finally, while biological nitrification inhibition under the plantain is suggested to be the primary mechanism responsible for the reduction in NO_3^- leaching losses from the diverse forage in Chapter 6, there was also an indirect benefit of reduced drainage under the diverse forge which was attributed to greater evapotranspiration rates by the plantain.

7.1.3 Effect of N loading rate and timing of urine deposition

Hypothesis #5: That NO_3^- leaching losses will be lower from urine deposited by cows grazing a diverse forage compared with cows grazing a standard forage containing perennial ryegrass and white clover.

A review of the literature has shown that urinary–N excretion is lower from cows grazing diverse forages which contain the herbs chicory and plantain (Woodward *et al.*, 2012; Edwards *et al.*, 2014; Box *et al.*, 2016; Cheng *et al.*, 2017). This has the potential to reduce NO_3^- leaching losses from cow urine patches (Li *et al.*, 2012). However, in Chapter 4, it was not possible to detect a statistically significant difference in NO_3^- leaching losses when cow urine was applied at a rate of 500 or 700 kg N ha^{-1}, thus hypothesis #5 should be rejected. One explanation for this conflicting result could be the low amount of NO_3^- leaching loss that occurred under <u>spring</u> applied urine (< 4 kg N ha^{-1} under optimum irrigation). Previous studies have shown that NO_3^- leaching decreased significantly with decreasing N loading rates when urine was applied in autumn (Di & Cameron, 2007). This suggests that there may be a greater role for diverse forages (with lower N loading rates) to reduce NO_3^- leaching losses from autumn applied urine, when plant growth rates are lower.

Hypothesis #6: That NO_3^- leaching losses will be lower from urine deposited during the early summer compared to late summer due to greater opportunity for plant N uptake over the summer period.

In Chapter 6, the amount of NO_3^- leached from late summer applied urine was higher than the amount of NO_3^- leached from early summer applied urine under the diverse forage. A similar trend was observed under the standard forage although this was not statistically significant in this experiment. Thus hypothesis #6 is correct for the diverse forage but not for the standard forage. The lower NO_3^- leaching losses from the early summer applied urine on the diverse forage compared with late summer applied urine reflected a longer period spent under favourable growing conditions which promoted N uptake. This is consistent with the low NO_3^- leaching (<4 kg N ha^{-1}) that was observed from spring deposited urine under optimum irrigation in Chapter 5. The results from both Chapters 5 & 6 have shown that under good irrigation management, NO_3^- leaching from spring and summer deposited urine is substantially lower than the NO_3^- leaching losses reported from autumn deposited urine (Cameron *et al.*, 2013; Selbie *et al.*, 2015). Irrigation, when managed correctly, therefore has an important role in increasing herbage N uptake from spring and summer deposited urine patches and subsequently reducing the risk of NO_3^- leaching loss during autumn and winter.

7.2 Conclusions

The strategic use of diverse forages containing plantain, is a viable mitigation option to reduce NO_3^- leaching losses from urine patch areas. Furthermore, the results have demonstrated that diverse forages can perform well under a range of irrigation types in New Zealand when irrigation is applied using best management practices. The main conclusions drawn from this PhD research project are:

- Irrigation can reduce NO_3^- leaching losses from spring deposited cow urine patches by 88-97% when irrigation is applied at an optimum rate for plant growth over the summer period.

- Under water limited condition such as deficit irrigation, N from spring deposited cow urine patches can remain in the soil where it is susceptible to leaching with the onset of drainage during the winter period.

- The population abundance of ammonia oxidizing bacteria in the soil is lower under diverse forages containing plantain compared with a standard perennial ryegrass and white clover forage. This is attributed to the release of biological nitrification inhibiting compounds into the soil by plantain.

- Diverse forages containing plantain can reduce NO_3^- leaching losses from summer deposited cow urine by 74-82% when compared with a standard perennial ryegrass and white clover forage. The lower NO_3^- leaching losses under the diverse forage were attributed to a combination of the release of biological nitrification inhibiting compounds into the soil by plantain and greater water use by plantain resulting in less drainage.

- The type of irrigation used (pivot, rotorainer or flood) had no effect on herbage DM yield, herbage N uptake or NO_3^- leaching losses from either the standard or the diverse forage types.

7.3 Limitations

Some of the limitations of this PhD research are:

- Both lysimeter experiments were conducted over one season. While this was necessary due to time constraints, large variation can occur between years in field experiments. This is due to a range of factors including differences in weather, climate, growing conditions and plant species persistence. The collection of long term data is therefore required to understand the full effects of diverse forage types and irrigation management on plant N uptake and NO_3^- leaching losses.

- The use of lysimeters to measure NO_3^- leaching under flood irrigation, where gradient effects were not accounted for. For example, under flood irrigated border dyke systems, the area closer to the irrigation channels receives a greater amount of water to ensure more distant locations get an adequate supply. The results of Chapter Six may therefore better represent the area closest to the irrigation channels rather than whole paddock conditions.

- The replication of the ^{15}N diffusions in Chapter Four was restricted to three replicates due to resources and the low concentrations of mineral N in the leachate.

- The replication of the soil blocks in Chapter Five was minimal due to resources and logistics (12 treatments × 4 replicates).

7.4 Suggestions for future research

This research project has highlighted the following key areas for future research:

- Further research is required in other areas in New Zealand with different soil types and climatic conditions. The experiments in this study were carried out on a Paparua fine sandy loam. It is expected that irrigation management and NO_3^- leaching losses would vary, on heavier or freer draining stony soils, which are also common throughout New Zealand.

- Quantifying the effects of irrigation and diverse forages containing plantain on NO_3^- leaching losses at other times of the year is also required. For example, how does deficit irrigation affect NO_3^- leaching losses from summer deposited urine patches or can diverse forages containing plantain reduce NO_3^- leaching losses from autumn deposited urine when temperatures are cooler?

- The proportion of plantain in diverse forages required to achieve biological nitrification inhibition needs to be quantified. This research project showed that NO_3^- leaching losses can be reduced when diverse forages contain approximately 20–30% plantain. It would be beneficial to determine if similar NO_3^- leaching losses occur under a lower proportion of plantain or can be reduced further if the proportion of plantain in the diverse forage is increased.

- There is also a need to identify the compounds in plantain responsible for biological nitrification inhibition in the soil and how these compounds affect ammonia oxidising bacteria. Through various plant breeding techniques and strategic genetic selection, it may be possible to develop cultivars capable of producing a greater amount of these compounds. Future research could also help determine if these compounds are excreted back onto the soil by animals grazing plantain, and any subsequent effects this may have on nitrification inhibition in the soil.

- Further research which determines the full fate of urinary deposited N under diverse forages containing plantain is also required. For example, gaseous N losses, N stored in plant roots, and N immobilised and remobilised throughout the season. Experiments that run over multiple seasons would also therefore be beneficial in quantifying N losses under diverse forages compared with standard perennial ryegrass and white clover forages.

Appendix A

Lincoln University Research Dairy Farm map

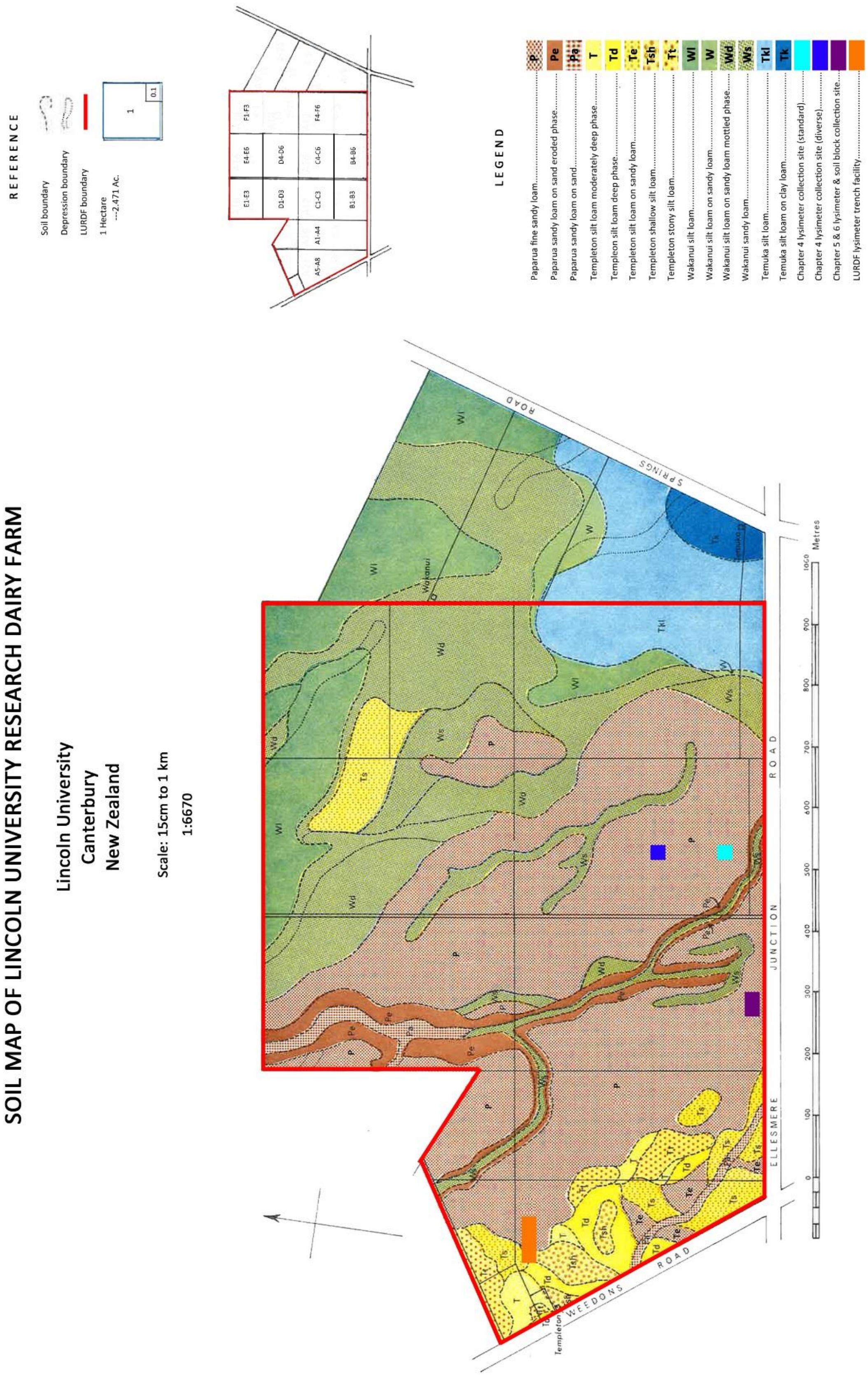


Figure A.1. Soil map of the Lincoln University Research Dairy Farm & the collection sites that lysimeters and soil blocks were taken from.

Appendix B

Chapter 6 supplementary soil data

B.1 Results

B.1.1 Soil nitrogen

Ammonium

At the end of the experiment, soil NH_4^+–N concentration ranged from 3.1–6.3 mg N kg soil^{-1} between 0–600 mm (Figure B.1). At 200–400 mm, the soil NH_4^+–N concentration under flood irrigation was significantly ($P < 0.05$) greater than pivot and rotorainer irrigation (February urine application) (Figure B.1). Forage type and urine application date had no effect on the soil NH_4^+–N concentrations (Table B.1).

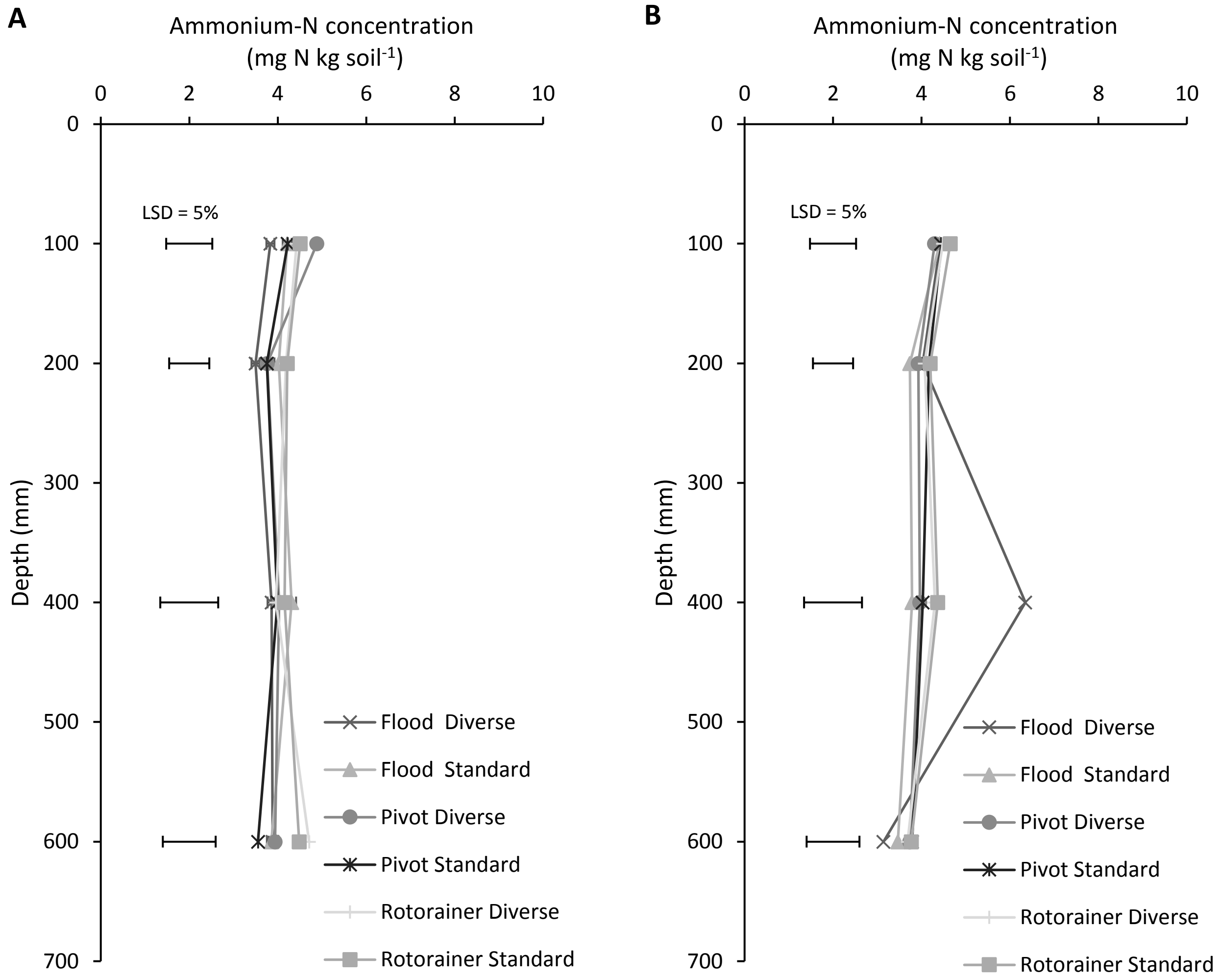


Figure B.1. Average soil ammonium concentration (mg N kg soil^{-1}) with depth at the end of the experiment from lysimeters as affected by irrigation (pivot vs. rotorainer vs. flood), forage type (standard vs. diverse) and urine application date (December (A) and February (B)). LSD (P <0.05) calculated from treatment means.

Nitrate

At the end of the experiment, averaged across all treatments, soil NO_3^-–N concentration was affected by forage type at 0–100 mm and 100–200 mm ($P < 0.001$), and irrigation at 100–200 mm ($P = 0.027$) (Table B.1). At 0–200 mm, soil NO_3^-–N concentration ranged from 0–0.32 mg N kg soil^{-1} (diverse forage) and 0.33–1.31 mg N kg soil^{-1} (standard forage) (Figure B.2). At 0–100 mm, the soil NO_3^-–N concentration under the standard forage was significantly ($P < 0.05$) greater than soil NO_3^-–N concentration under the diverse forage for all irrigation treatments (December urine application). At 0–100 mm, the soil NO_3^-–N concentration under the standard forage was significantly ($P < 0.05$) greater than soil NO_3^-–N concentration of the diverse forage for pivot and rotorainer irrigation treatments (February urine application) (Figure B.2). A similar trend was observed at 100–200 mm.

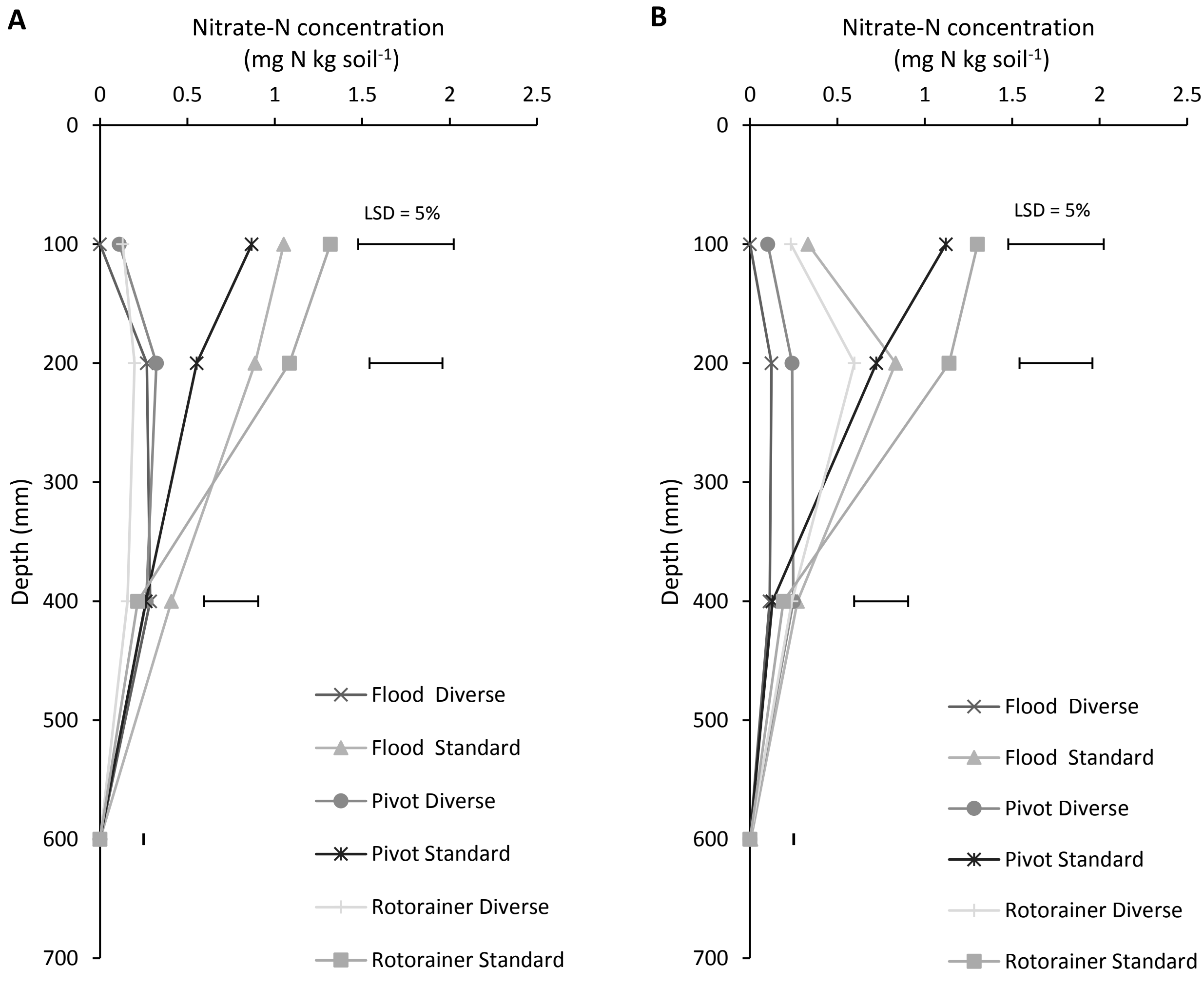


Figure B.2. Average soil nitrate concentration (mg N kg soil^{-1}) with depth at the end of the experiment from lysimeters as affected by irrigation (pivot vs. rotorainer vs. flood), forage type (standard vs. diverse) and urine application date (December (A) and February (B)). LSD (P <0.05) calculated from treatment means.

Total nitrogen

At the end of the experiment, averaged across all treatments, soil total N concentration was affected by forage type at 0–100 mm (P < 0.020) (Table B.1). Total soil N was greatest at 0–100 mm, ranging from 0.18–0.20% and decreased with depth (Figure B.3).

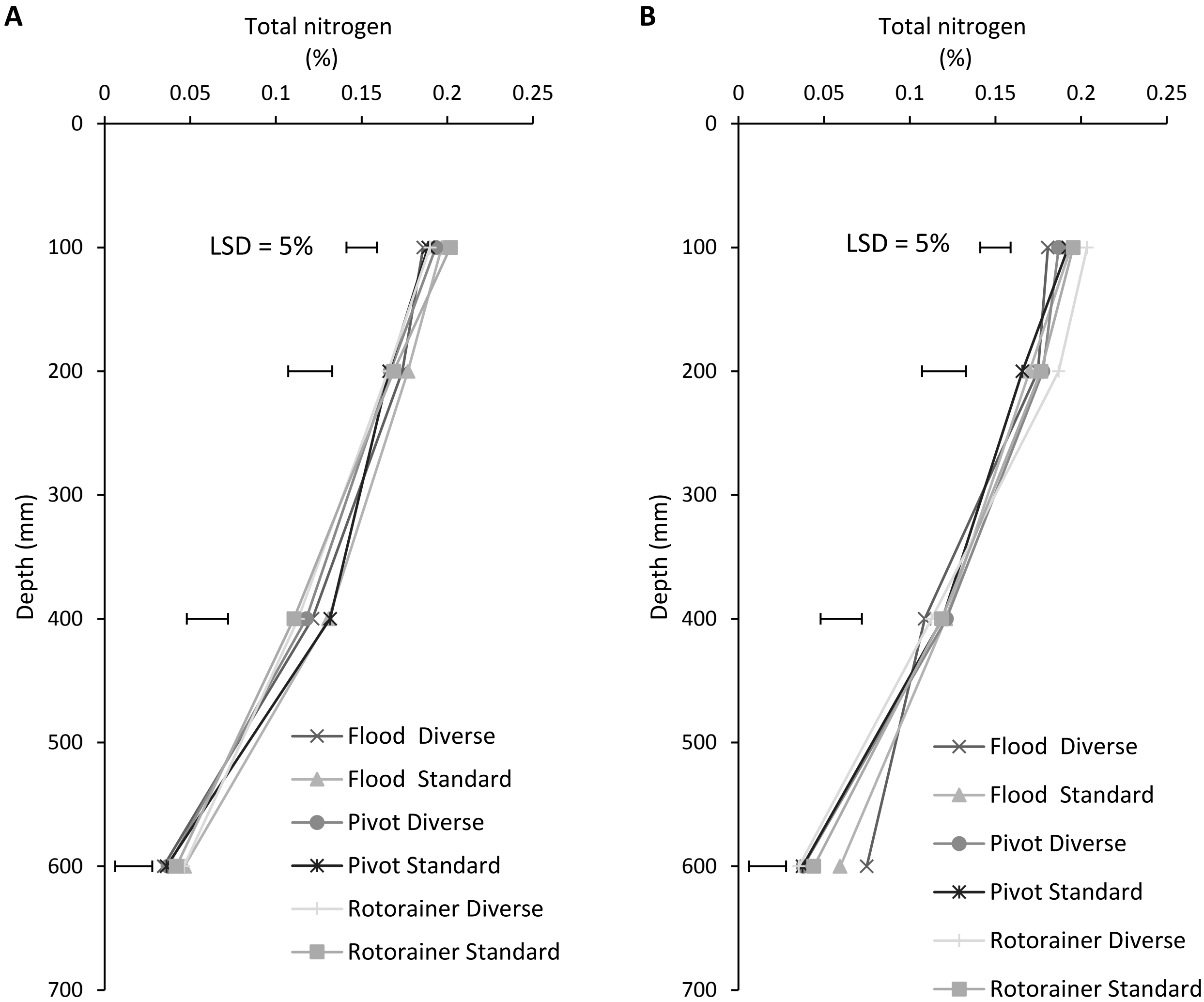


Figure B.3. Total soil N with depth at the end of the experiment from lysimeters as affected by irrigation (pivot vs. rotorainer vs. flood), forage type (standard vs. diverse) and urine application date (December (A) and February (B)). LSD (P <0.05) calculated from treatment means.

Table B.1. Soil ammonium and nitrate concentrations (mg N kg soil^{-1}), and total soil N (%) at depths of 0-100 mm, 100-200 mm, 200-400 mm and 400-600 mm from lysimeters at the end of the experimental period.

Irrigation	Forage	Urine rate	Soil ammonium N concentration (mg N kg soil^{-1})				Soil nitrate N concentration (mg N kg soil^{-1})				Soil total N (%)			
			0-100	100-200	200-400	400-600	0-100	100-200	200-400	400-600	0-100	100-200	200-400	400-600
Pivot	Diverse	December	4.886	3.763	4.03	3.94	0.110	0.323	0.263	0.000	0.193	0.167	0.118	0.037
	Standard	December	4.163	3.837	3.94	3.59	0.895	0.545	0.220	0.000	0.195	0.172	0.125	0.037
	Diverse	February	4.297	3.928	3.96	3.74	0.102	0.241	0.248	0.000	0.187	0.179	0.121	0.038
	Standard	February	4.387	4.250	3.97	3.83	1.148	0.751	0.080	0.000	0.198	0.172	0.113	0.038
Rotorainer	Diverse	December	4.426	4.189	3.96	4.71	0.130	0.198	0.157	0.001	0.190	0.165	0.113	0.046
	Standard	December	4.508	4.216	4.16	4.48	1.318	1.084	0.216	0.000	0.202	0.169	0.111	0.042
	Diverse	February	4.440	4.034	4.46	3.95	0.231	0.682	0.297	0.000	0.204	0.188	0.114	0.034
	Standard	February	4.649	4.202	4.37	3.77	1.302	1.139	0.191	0.000	0.195	0.177	0.119	0.044
Flood	Diverse	December	3.836	3.499	3.86	3.89	0.000	0.267	0.287	0.000	0.181	0.174	0.121	0.043
	Standard	December	4.215	4.028	4.31	3.86	1.051	0.887	0.408	0.000	0.197	0.177	0.131	0.047
	Diverse	February	4.272	3.849	6.17	3.12	0.012	0.048	0.072	0.000	0.184	0.182	0.117	0.075
	Standard	February	4.410	3.740	3.79	3.47	0.327	0.833	0.270	0.005	0.194	0.170	0.121	0.059
LSD (5%) within irrigation regimes			0.845	0.821	1.29	1.03	0.507	0.424	0.261	0.005	0.014	0.019	0.030	0.023
LSD (5%) for all other comparisons			1.169	0.907	1.31	1.20	0.546	0.417	0.309	0.005	0.018	0.026	0.032	0.026
<u>Significance of main effect</u>														
Irrigation			NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
Forage			NS	NS	NS	NS	***	***	NS	NS	*	NS	NS	NS
Urine app. month			NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS
<u>Significance of interaction</u>														
Irrigation × forage			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Irrigation × urine app. month			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Forage × urine app. month			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Irrigation × forage × urine app. month			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

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